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An Experimental Study of Aperture as a Factor in Microscopic Vision.

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It is a well known fact that the image of a point projected optically is not a point, but always a disc. The diameter of the disc varies directly with the uncorrected spherical and chromatic aberrations, and inversely with the aperture, of the projecting lens. If it were possible to fully correct spherical and chromatic aberrations, the image of a point would still be a disc varying in size inversely with aperture. Microscopists have long been familiar with the effect of spherical and chromatic aberrations in the microscope. But the effect of aperture as a factor in microscopic vision is not generally well understood and is worthy, I think, an hour's consideration by this society.

The practical importance of aperture in its relation to the resolving power of the microscope has been recognized longer than any explanation of that relation has been acceptable. Considered theoretically and independently as a factor in microscopic vision aperture has been almost ignored ; although as an associate factor, associated with diffraction by the finer details of microscopic objects, it has received no little attention. In fact, the final associated effects (in the projected image) of aperture and of diffraction of light in the plane of the object are the basis of the Abbe theory of microscopic vision. This theory and its easily repeated experimental study are fascinating and convincing. Since its announcement the Abbe theory has been for the most part accepted as rapidly as it has received serious attention. Nevertheless,

*Revised, extended and newly illustrated for the TRANSACTIONS of the Society.

a few men* while appreciating the labors of Professor Abbe and recognizing the importance in some ways of his discovery have called attention to unsatisfactory points in the theory. The writer, if he may, will submit for consideration these and some other points and a theory of microscopic vision in harmony with an experimental study of aperture as a factor in microscopic vision.

The Abbe theory is said to be applicable only to microscopic vision, to the projection of an image by a microscope objective and not to image projection by a telescope objective or any other variety of projecting lenses. On the other hand, it is said that the theory applicable to the formation of an image by other projecting lenses is not sufficient to account for all the effects seen in an image projected by a microscope objective. Is not such exclusiveness, or limitation, unusual in the application of a theory to one particular function performed by essentially the same instrument (*i. e.* a projecting lens), although under somewhat different circumstances?

The writer believes that the theory of the effect of aperture as a factor in microscopic vision is applicable to all projecting lenses, explaining resolving power and its limitation; that the diffraction of light by an object is to be considered in the same category with other changes in direction in incident light produced by an object, as, for instances, those resulting from reflection and refraction; that diffracted and other rays leaving an object in changed directions, as well as rays directly transmitted, when traveling the same paths between an object and the objective are affected alike by aperture; and that the final effects in the image experimentally studied by Professor Abbe are the result of changes above the objective due to aperture, and not to changes below the objective resulting from diffraction by the finer details of an object.

A few years ago a portrait lens served the writer in showing the Abbe diffraction phenomena to a number of physicians met to listen to a paper on the theory of microscopic

*Particularly Mr. E. M. Nelson in *The Journal of the Quekett Microscopical Club*, July, 1890, and March, 1895; Mr. B. Tompson Lowne in the same *Journal*, April, 1889; and Mr. Lewis Wright in the *English Mechanic*, Vol. LX [1894].

vision. The phenomena were the same as those with which most microscopists are familiar experimentally or by reading, and of which an authoritative account (*q. v.*) is given in the Dallinger edition of Carpenter's *Microscope and Its Revelations*. The portrait lens having a diameter of two inches and a focus of eight inches exhibited the phenomena on a large scale as well as a microscope objective exhibits the same phenomena on a smaller scale.

Early this month [August, 1896,] a parallel experiment was tried, using instead of the portrait lens a telescope objective having a diameter of two inches and a half and a focus of forty-three inches. An eye-piece was supported about sixty feet from the objective (*i. e.* the distance corresponding to tube length was about sixty feet, an actual tube of that length being unnecessary because the experiment was tried in dark rooms). The source of light was an electric arc lamp about twenty-seven feet in front of the objective. The object was a series of vertical lines scratched with a fine needle point through the opaque film of an old dry plate negative supported nearly forty-six inches in front of the objective. After this gigantic microscope was focused so as to show the lines through the eye-piece, a plane was found not far from the eye end of the actual telescope tube in which was a central image of the electric arc with a series of diffraction images on each side. These images could be dealt with by means of diaphragms so as to vary the final image seen through the eye-piece as one deals with the corresponding images ["spectra"] at the back of the microscope objective to produce changes in the final image of the ordinary microscope.

The scratched lines in the latter experiment were about one-thousandth of an inch broad and from one-hundredth to one-sixtieth of an inch apart. The least of these measurements is more than a few multiples of any wave length of light. Since the publication of his original paper Professor Abbe has changed his views in respect to the size of objects to which the diffraction phenomena and the associated image

changes are peculiar. He no longer holds that they are peculiar to objects measuring less than a few multiples of a wave length of light; for he has seen them produced by "gratings of not more than forty lines to the inch,"* seen through a lens of twelve inches focus.

If the Abbe diffraction phenomena and associated image changes can be experimentally demonstrated with a portrait lens or a telescope objective, is it not probable that they can be demonstrated with any projecting lens and, moreover, have any explanatory relation to the formation of the visual image in these cases that they have to the formation of the microscopic visual image? Again, if a portrait lens and a telescope objective behave in the same way as a microscope objective in experimenting with these diffraction phenomena and associated image changes, does a reason based upon such experiments exist for regarding microscopic vision as "*sui generis*"!†

There are three ways in which the projection of an image by a lens may be studied: One based upon the electromagnetic theory of stresses; a second, the physical, based upon the wave theory; and a third, the dioptric or geometrical, based upon the propagation of light in straight lines and the laws of reflection and refraction. The first has not been sufficiently studied to serve our purpose. The second, according to the Abbe theory, is the only one by which microscopic vision can be fully explained. The third which serves to explain the projection of images of macroscopic and larger microscopic objects is inadequate, according to the Abbe theory, to explain the projection of images of more minute microscopic objects.

A mixed method, largely the third and in part the second, is commonly used and is the "geometrical optics" of Lord Rayleigh and Professor Tait in the *Encyclopædia Britannica.* Under "geometrical optics" Lord Rayleigh not only includes "conceptions of the wave theory," but treats of "aperture"

* THE MICROSCOPE AND ITS REVELATIONS, Dallinger-Carpenter Edition, 1891, p. 64.
† *Ibid.*, p. 62.

and "resolving power."* "Indeed," Lord Rayleigh says,† "it is not to be denied that the too rigid separation of optics into geometrical and physical has done a good deal of harm, much that is essential to a proper comprehension of the subject having fallen between the two stools." Under this interpretation of geometrical optics the writer believes that the projection of images of the more minute microscopic objects can be fully explained and, moreover, that such an explanation in some shape is a necessary part of a complete theory of the formation of images by every projecting lens.

For a time our discussion can be simplified by getting rid of the idea of the actual sizes of objects seen through the microscope or the telescope. When objects and distances between objects are measured not by linear units but by the angles they subtend at the center of the objective, "resolving power" in the microscope is seen to be the same as "separating power" in the telescope. Resolving power increases with "twice the sine of half the angle of aperture" of the microscope objective. Separating power increases with the diameter of the telescope objective. But twice the sine of half the angle of the aperture of a given lens is its effective diameter. The same power, then, is required to show as separate pictures the images of two points of detail subtending an arc of one minute whether the actual distance between the points be very little, measured on the stage of the microscope, or very great measured among the stars. Resolving power may be regarded as separating power brought to bear upon an object near a microscope objective and separating power as resolving power brought to bear on an object a great distance from a telescope objective.

In Fig. 1, let A and B be isolated centers of motion of equal intensity radiating yellow light. Let SN be a section of a screen interrupting light from the points A and B. Let the rays of light Ac and Bc be equal in length. Let the ray Ad be shorter than the ray Bd by half a wave length of yellow

* ENCYCLOPÆDIA BRITANNICA, Ninth Edition, Vol. XVII., p. 806.

† *Ibid.*, p. 798.

light, *Ae* a whole wave length shorter than *Be*, *Af* a wave length and a half shorter than *Bf*, and *Ag* two wave lengths shorter than *Bg*. At *c*, *e* and *g* where whole waves of the incident rays coincide crest with crest and trough with trough the intensity of one ray is added to the other. At the intermediate points *d* and *f* where crests oppose troughs and troughs oppose crests motion becomes null and darkness results. The increased intensity at *c*, *e* and *g* is not limited strictly to these points in the line *SN* but is spread out upward and downward to a certain extent and then fades into the intervals of darkness. This is because crests nearly coïncide with crests at first, when the difference in the lengths of the uniting rays is still nearly a wave length or an integral multiple of a wave length. But as waves fail more and more to coincide crest with crest and trough with trough and tend more and more to oppose troughs to crests and crests to troughs, interference results in less and less motion and consequently less and less light.

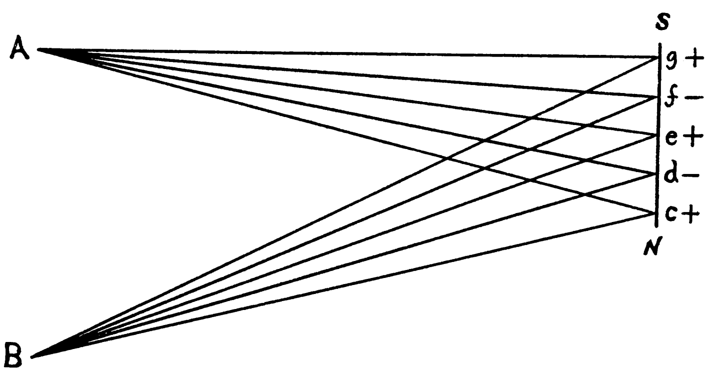


FIG. 1.

The distances between the points of greatest intensity *c*, *e* and *g* are seen to depend upon the wave length of yellow light. If the centers *A* and *B* radiated red light, with a longer wave length, the distances between the points of greatest intensity on the screen would be greater and the upward and downward spreading of light would be more

correspondingly. On the other hand, if the centers *A* and *B* radiated blue or violet light, with a shorter wave length, the distances between the points of greatest intensity would be less and the upward and downward spreading of light reduced correspondingly.

If the screen were nearer or farther from *A* and *B*, it would present the same appearance with this exception ; that the distances between the points *c*, *d*, *e*, *f* and *g* would vary with the distance between the sources of light and the screen. If the line *SN* of the screen were longer, additional points of darkness and light would appear above *g* and below *c*. [In this and the diagrams about to be used the wave length is thousands of times longer than an actual wave length of light ; so the distances between such points as *c* and *d*, or *c* and *e*, are thousands of times too great, made so for purposes of demonstration.]

In Fig. 2, let *ABCDE* be a section of the emitting surface of a projecting lens. Let *O* be a self luminous object (or one illuminated with a full cone of sub-stage light) subtending a small angle, *bCc*, at the center of the lens. Let *OA*, *OB*, *OC*, *OD* and *OE* be rays which radiate from a central isolated point in the object *O*, pass through a first lens surface not shown and reach the emitting lens surface *AE*. Let *SN* be a section of a screen receiving the projected image of a central isolated point in the object *O*. In addition to the refracted primary rays *Ag*, *Bg*, *Dg*, *Eg* and the direct primary ray *Cg*, other less intense secondary rays*—such as those shown by broken lines—are also propagated from the lens to the screen, the secondary rays having less intensity with their obliquity to the primary ray from which they radiate. Such radiating secondary rays originate at every emitting point of the lens surface *AE*. Secondary rays from two isolated points, as *B* and *C*, when interrupted by the screen *SN*, would behave as behaved the radiating rays in Fig. 1.

The full aperture of the lens is *AE*. Suppose this aper-

*For an account of the origin and behavior of secondary waves, or rays, the reader is referred to Preston's THEORY OF LIGHT, Chapters III, VII, VIII and IX.

ture to be reduced one-half, the emitting surface *AE* reduced to *BD*. Thus the points of emission *A* and *E* are excluded by this first supposition. Let *B*, *C* and *D* be isolated points of emission, *B* and *D* being at opposite edges of the aperture.

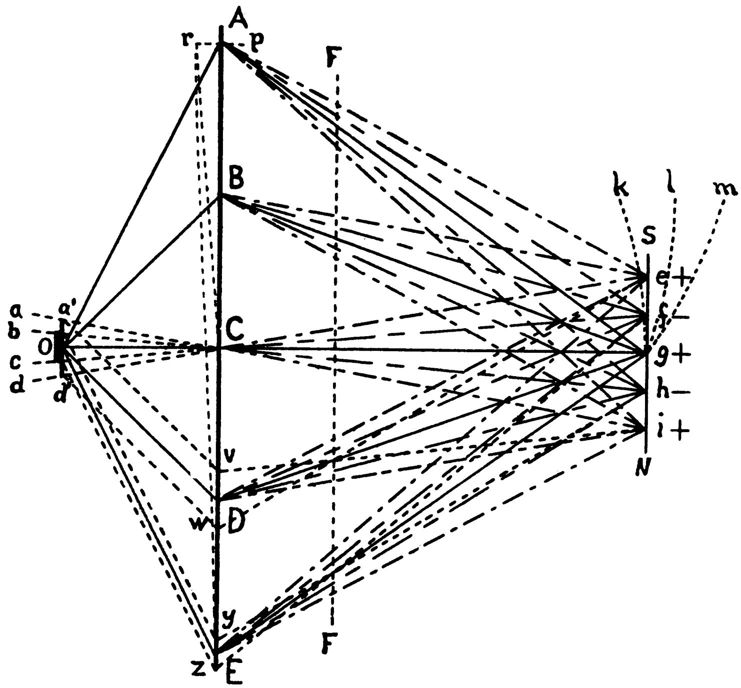


FIG. 2.

Let the rays *Bf* and *Dh* be longer than the rays *Cf* and *Ch* by half a given wave length. Let the rays *Be* and *Di* have the same length as the rays *Ce* and *Ci*. Let the ray *Bi* be longer than the rays *Ci* and *Di* by two wave lengths and the ray *De* be longer than the rays *Ce* and *Be* by two wave lengths. As a result, the points *e*, *g* and *i* in the line *SN* are illuminated and the points *f* and *h* are dark. Spreading of light occurs at *e*, *g* and *i*. If the screen were more extended, additional points of light would be seen above *e*, and below *i*. What occurs in the line *SN*, one diameter of the screen, occurs in all diameters. Consequently *g* becomes

a disc of light with a fading peripheral zone while *f* and *h* become a ring of darkness and *e* and *i* a ring of light. If the screen were more extended, larger and concentric rings of darkness and light would be added to the pattern. The intensity of the smallest ring of light would be much less than that of the center of the disc, and with increasing diameter the intensity of each ring would rapidly diminish to invisibility.

If, now, the object *O* be an unbroken surface and self luminous (or illuminated with a full cone of sub-stage light), lying between the dotted lines *bC* and *cC*, then its diameter would be made up of a line of luminous points each of which would radiate light to the projecting lens as did the central point we have considered. One ray from each point would pass directly through the center of the lens and fall upon the screen *SN* somewhere between the points *f* and *h*. Each of these direct primary rays would at its point of emission *C* in the lens surface *BCD* give origin to radiating secondary rays. One ray also from each point would after refraction by the lens leave the emitting surface at *B* and fall upon the screen somewhere between the points *f* and *h*. Each of these refracted primary rays would at its point of emission *B* give origin to radiating secondary rays. So, also, corresponding primary refracted rays would be emitted from *D* and fall upon the screen between *f* and *h*, each giving origin on emission to similar radiating secondary rays. Then might *Ce* be regarded as a secondary ray originated at *C* from a primary ray in the path *cCf*, and *Ci* a secondary ray from a primary ray in the path *bCh;* and *e* would now have the same relation to *f*, and *i* to *h*, as we saw a moment ago *f*, or *h*, had to *g*. *f* and *h* would now be bright ends of the image *fh* of the diameter of the object *O;* and the points *e* and *i* would be dark by interference, while spreading of light from *f* toward *e*, and from *h* toward *i*, would occur. This spreading of light from *f* upward and from *h* downward results in elongating the image of the diameter of the object. [A perfect dioptric image would be sharply brought to an end at *f* above

and h below.] Were the screen SN a little more extended downward, a bright point would appear below i and would have the same relation to h that we saw a moment ago i, or e, had to g. A corresponding point would appear above e, if the screen were extended a little upward. What would be true in the projection of an image of one diameter of an object would be true in the projection of an image of every other diameter. Consequently, the surface of the object O, supposed to be round, would be projected on the screen SN as a disc having a diameter a little greater than fh and not a sharp but a fading, or fluffy, boundary. Were the object a line and O between bC and cC its section, then the image would be a band of which the diameter image already described would be a section. Were the screen more extended, the disc would have concentric rings about it, or the band would be flanked above and below by parallel lines. (See first the dots and lines a, i and g in Photo. 1, and then the same dots and lines in Photo. 6.)

It will be observed that in the first instance (the projection of an image of a central isolated point) e and i were bright and in the second (the projection of an unbroken surface) dark. In either case the illumination or darkness was the effect of the disturbance at e or i resulting from the union of the wave motions of all the rays there incident. If in the first instance all the points in the diameter BCD were simultaneously effective, the illumination at e would be the result not only of the union of coincident crests and troughs of the secondary rays Be, Ce and De, but also of a like union, pair by pair, of one secondary ray from each succeeding point of emission in the lens surface downward from B to C with one secondary ray from each corresponding succeeding point of emission downward from C to D. The illumination at i would be the result of a similar union of the secondary rays Bi, Ci and Di and also the union, pair by pair, of secondary rays from the points between B and C with secondary rays from points between C and D. (A primary ray is indicated by an unbroken line, as Cg or Bg; a secondary ray of first intensity,

by a broken line of long portions, as *Bf* or *Bh* ; and a secondary ray of second intensity, by a broken line of shorter portions, as *Be* or *Bi*.

If in the second instance all the points in the diameter *BCD* were simultaneously effective, the darkness at *e* would be the result not only of the union of *Be*, *Ce* and *De*, opposed in phase, but also a like union, pair by pair, of one secondary ray from each succeeding point of emission in the lens surface downward from *B* to *C* with one secondary ray from each corresponding succeeding point of emission downward from *C* to *D*. Darkness at *i* would be accounted for in a similar way. What would be true in this and the former instance in one diameter would be true in all diameters of the same surface.

Each lens surface in an objective gives rise to secondary rays and is also a reflecting as well as a refracting surface. So the primary rays incident at the last surface of an objective are ray by ray the compounded resultants of the union of direct, reflected and secondary rays (less energy by giving rise to secondary rays and by reflection) at all previous transmitting surfaces. The total result in a path leaving the last surface is compounded wave motion which on being interrupted by a screen unites with other resultant compounded waves arriving by different paths at the same point in the screen. But the primary rays in their paths through the objective are affected essentially alike ; so that those which leave any one point in an objective arrive at the image of that point in the same phase. A primary ray determines the phase of the secondary rays to which it gives origin at the last surface of the objective. Thus the phase of the compounded wave motion of the ray *Be* is determined in the second instance chiefly by the phase the primary ray *Bf* at the emitting point *B*; the phase of *Ce*, chiefly by that of *Cf* at *C*; and the phase of *De*, chiefly by that of *Df* at *D*.

Now let the full aperture *AE* in Fig. 2 be effective. Let an image of a central isolated point of the object *O* be projected by the lens upon the screen *SN*. By the doubling of aperture, the lens not only receives additional and more

oblique rays from the object, but has additional points of emission. *OA* is one of the additional and more oblique rays from the object. *A* and *E*, at opposite edges of the double aperture, are additional points of emission. After refraction the ray *OA* takes the path *Ag*, which is evidently longer than the path *Bg* taken by the ray *OB* after refraction. Both these paths are longer than the path *Cg* taken by the ray *OC* after transmission.

Let the arc *gm* be drawn with *Ag* as a radius; the arc *gl*, with *Bg* as a radius; and the arc *gk*, with *Cg* as a radius. If the *g* ends of the first two radii were swung upward in the arcs *gm* and *gl*, the interrupting screen *SN* would shorten the radius *Ag* more rapidly than it would the radius *Bg* (relatively as are the distances of the arcs *gm* and *gl* from the screen *SN*). If the *g* end of the radius *Cg* were swung upward in the arc *gk*, it would have to be longer to reach the screen. This addition in length to enable it to reach a given point in the screen would be less than the shortening the radius *Bg* or *Ag* would suffer if interrupted at the same point. It becomes evident diagrammatically that were the *g* ends of these radii swung upward in the described arcs, the difference in length between the radii *Ag* and *Cg* would amount to half a wave length in a swing about half as great as the swing it would be necessary to give the *g* ends of *Bg* and *Cg* to make the latter differ in length by half a wave length. As a result, such darkness as the aperture *BD* placed at *f* would, by the double aperture *AE*, be placed half way between *g* and *f*. In a similar way, such illumination as the smaller aperture placed at *e* would, by the larger aperture, be placed at *f*. In a corresponding way, the spreading of light upward from *g* would be reduced to half what it was with the half aperture. Symmetrical phenomena would occur below the axis of the lens. What is true of one diameter of the screen is true of all its diameters. Therefore, the image of a central isolated point in an object is projected by a lens of given aperture as a disc and concentric rings having diameters equal to half those of the disc and rings in the image projected by a lens of half

the given aperture. A similar study of other apertures would but add to this evidence showing that the interference or diffraction pattern, disc and rings, is proportionally contracted with every increase of aperture.

Not only have microscopists noticed in practice the direct relation of aperture to resolution, but also the fact that isolated lines or particles in an object appear broader through an objective of small aperture and narrower through an objective of large aperture. This narrowing effect of increasing aperture is due to the contraction of the diffraction pattern. *It is easily understood that the projected image discs of a series of close points in an object (or the projected image bands of a series of close lines) might touch or overlap when projected by a lens of small aperture, and, on the other hand, might be separated or resolved when projected by a lens of sufficiently large aperture.* The separating or resolving power of the telescope is thus explained. The same explanation has been applied, by inference at least, to projecting lenses generally, not excluding the microscope. Only Professor Abbe and his followers exclude the microscope and claim that microscopic vision is "*sui generis.*"

Simple parallel experiments with the telescope and microscope show that the actual effects of aperture in both instruments are in harmony with the above explanation:

Experiment 1: The instrument used was a telescope having an aperture of two inches and a half and a focus of forty-three inches, standing twenty-seven feet from a window in a darkened room. Outside the window was a mirror reflecting light from a bright sky into the room. Of all the light reflected from the mirror that only reached the telescope which passed through two pinholes in a piece of black paper supported in front of the mirror. The diameters of the pinholes were one-thirtieth and one-twentieth of an inch respectively; and the distance between them, one-tenth of an inch. The iris diaphragm of an Abbe sub-stage condenser was supported centrally in a temporary mounting of wood fitting into the hood of the objective.

When the diameter of the iris opening was one-sixteenth of an inch, the two pinholes appeared, when seen through the telescope, as one dim hazy disc. When the diameter was one-eighth of an inch, a smaller and more distinct disc was seen. When the diameter was three-sixteenths of an inch, the disc was still smaller, brighter and better defined—with a dim, hazy overlapping disc becoming evident. When the diameter was one-fourth of an inch, the discs (now distinctly two) were smaller, brighter and just separated. When the diameter was three-eighths of an inch, both discs were brilliant and well separated, their relative sizes and distance apart approaching truth. When the diameter was one-half an inch, the picture was more brilliant—the larger disc tending to appear star-like with irradiation. With the full aperture of two inches and a half irradiation was marked in both. During these observations thin concentric circles of light were glimpsed.

Experiment 2: The instrument and all the conditions excepting one remained the same. The exception was this: in the hood was fitted a piece of stiff black paper instead of the iris diaphragm, the circular piece of paper allowing no light to enter the objective except that which passed through a slot corresponding with one of its diameters. Thus the objective was made rectangular in shape with a narrow aperture in one direction and a long, or wide, aperture in the other.

The piece of paper with its slot was turned about so that the slot corresponded with various diameters of the hood. The discs seen through the telescope appeared stretched out, as it were, into lines always crossing at an angle of 90° the diameter of the instrument corresponding with the slot. The width of each line was determined by the long aperture; the length, by the narrow aperture. A comparison of the width of each line with its length showed the comparative effect of the two apertures in contracting the diffraction pattern.

Experiment 3: The instrument used was a Powell and Lealand microscope with a centering and focusing sub-stage. The objective was a Zeiss "aa", having a focus of about 1-inch, and N. A. of .17. The eye-piece was usually a Powell

and Lealand "compensating 20." The sub-stage carried a Powell and Lealand apochromatic condenser of $\frac{1}{4}$-inch focus, and N. A. of about .9. At a distance of three feet in front of the microscope the same pinholes used in the first two experiments were arranged so as to allow only such light from a lamp flame as passed through them to reach the mirror of the microscope. The light reaching the mirror was reflected through the sub-stage condenser to an aërial image of the pinholes projected by the condenser in the plane of the microscope stage.

The aërial image of the pinholes was the object observed through the microscope. Seen with a small diaphragm opening back of the objective the pinholes appeared as two discs just touching one another. With larger openings the discs became smaller, more brilliant and separated. The effects of varying aperture (varied by means of diaphragm openings back of the objective) in this experiment with the microscope were the same as those seen in the first experiment when aperture was varied by means of diaphragm openings in front of the telescoe objective.

Experiment 4: The instrument and all the conditions excepting one remained the same as in the third experiment. Instead of the central opening in a diaphragm back of the objective, a slot corresponding to one diameter was used. The aperture of the objective thus became rectangular in shape.

The image of the pinholes was observed while the slot was turned so as to lie successively in all diameters of the instrument. The effects were the same as those seen when the corresponding Experiment 2 was made with a telescope. Before the diaphragm was placed back of the objective the discs appeared as shown diagrammatically in Fig. 3 at *a* and *b*. Later, with the slot directed as shown at *A*, the image became a line for each disc (a' and b') with diffraction points at each end, the latter not shown in the figure. With the slot directed as shown at *B* the diffraction patterns overlapped (a'' and b''). a' and b' show resolution by wide aperture (the

length of the rectangular aperture); and a'' and b'', failure in resolution by narrow aperture. With a slot three times as wide the lines a' and b' become one-third as long; and a'' and b'' did not overlap, but appeared as shown at a''' and b'''. Resolution in the last instance was affected by both apertures, but to a greater degree by the longer.

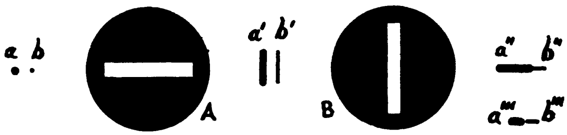


FIG. 3.

Resolution and failure in resolution are also shown at H and D in Photo. 25, taken with a horizontal slot, and at H and D in Photo. 26 taken with the same slot placed vertically, the object and other conditions in each case being the same. In these two Photo's are seen faint diffraction points at the ends of some lines [well shown in the photomicrographs, but not in the half-tone reproductions] and faint diffraction lines flanking others, depending upon the direction of the slot.

Our geometrical and experimental study thus far suggests the following:

A perfectly projected dioptric image [one free from defects due to spherical and chromatic aberrations and diffraction] would be point by point a correct picture of an object. But as the images of points are not projected as points, but as discs, a perfectly projected dioptric image is an impossibility. Excluding spherical and chromatic aberrations, these discs (in so much that they are larger than points) are due to aperture diffraction effects. Aperture diffraction effects are contracted with every increase of aperture. *The essential object of increasing aperture is to contract diffraction patterns, and thus cause the projected image to approach as a picture as nearly as possible that which we can only imagine, never realize, a perfect dioptric image.* The more nearly the picture approaches in character a dioptric image, the less will be the overlapping of the projected images of details, the greater will be the

separating or resolving power of the projecting lens, and the better will be our idea of the object under observation.

This is illustrated by Photo's 9 and 10. Photo. 9 was taken with an aperture barely wide enough to resolve the closer of a double series of lines cut through an opaque film of silver. The bare resolution of the closer lines gives us a poor idea of fine cuts in an opaque film. We need to see the details of the edges of the lines and of appearances between the lines (as in Photo. 10), requiring wider aperture in various diameters, to get a better idea of cuts and imperfections in the film. *We need then wider and wider aperture to resolve details of details, and wider and wider aperture still in every diameter to resolve successively finer and finer details of details (as long as they are present, or a practical limit to increase of aperture is not reached). This is accomplished by contracting more and more the diffraction disc image of each of the individual points of which every object may be considered to be an aggregation, be its form what it may.* Not only does our study thus far suggest the foregoing theory of microscopic vision, one applicable to all projecting lenses, but calls attention to the fact that one of the essential factors of the Abbe theory is not always present; for *the details of our self-luminous aërial microscopic object originated no diffracted rays*, although the microscope objective projected and resolved in usual fashion.

Suppose, for a moment, our aërial object originated diffracted rays. As every path between the object and the objective was occupied by a primary ray, diffracted rays could have taken only the same paths. Starting from the same points in the object they could have had no individuality; for each, diffracted from one primary ray, would have become, at its very origin, a part of another primary ray leaving the same point—just making up for the loss by the latter of a corresponding diffracted ray given to the former. A similar exchange of diffracted rays everywhere* in the cone would

*"Everywhere" is not strictly true when a cone just fills an objective. Primary rays toward and at the periphery would then lose rays diffracted outward beyond the limit of the cone and would receive no diffracted rays in return, as there would be no primary rays outside the cone from which diffracted rays could originate.

have resulted in primary rays not differing appreciably from those which occupied the same paths between the object and objective, diffraction not occurring. Such were the conditions under which Photo's 9 and 10 were taken. The irregular rectangular figure [the same as *B* in Photo. 1} is the area of the source of light projected in the plane of the film of silver, projected by a sub-stage condenser cone of light of more than sufficient angle to send primary rays along every path between the object and the objective. Diffraction by the cuts and imperfections in the film resulted in an interchange of diffracted. rays, but no appreciable alteration in the character of the primary rays, *i. e. diffracted rays from an object lose individuality at their very origin when an objective is filled by a full cone of light from a sub-stage condenser.* Under second conditions then we find a factor essential to the Abbe theory of microscopic vision is not necessary; *for Photo's 9 and 10 show the effects of different aperture notwithstanding diffraction by the details of the object was practically nullified by full cone illumination.*

That rays diffracted by an object are practically nullified by full cone illumination can be shown experimentally. While the apparatus was arranged for taking Photo. 9, the full cone of light from the sub-stage condenser was reduced to a small axial pencil. A bit of white card was held against the front of the objective. Each cut in the film diffracted light, spreading obliquely away from the axial pencil. The diffracted rays leaving different cuts and becoming incident at any one particular point on the card traveled paths of different lengths. Interference phenomena occurred. Any pair of adjacent cuts had essentially the same relation to the card that the two sources of light *A* and *B* in Fig. 1 had to the screen *SN*. The result on the card was a bright round spot of light (illuminated by the axial pencil) flanked on either side by fainter repetitions of the round spot corresponding in their manner of production with *e* and *g* in Fig. 1. The fainter spots were formed wholly by diffracted rays from the cuts. A full cone from

the sub-stage condenser was then used again. Primary rays once more traveled every path between the cuts and the card, and engulfed, as it were, the diffracted rays. The card was illuminated by one large circular spot, including and blotting out all the previous picture.

Lord Rayleigh, writing of resolving power of optical instruments in general, and of the telescope in particular, says: "The contraction of the diffraction pattern with increase of aperture is of fundamental importance with reference to the resolving power of optical instruments"*; resolving power is "proportional to the aperture, and independent of focal length"†; and "The theory of resolving power is rather simpler when the aperture is rectangular instead of circular, and when the subject of examination consists of two or more light or dark lines parallel to one of the sides of the aperture. Supposing this side to be vertical, we may say that resolving power is *independent of the vertical aperture*, and that a double line will be about on the point of resolution when its components subtend an angle equal to that subtended by the wave length of light at a distance equal to the *horizontal aperture*."‡ Lord Rayleigh § is also authority for the experimental result that, if for a rectangular aperture under the conditions just quoted a round aperture be substituted, the latter to have the same resolving power must have a width or diameter about ten per cent greater. Sir George Airy¶ has found by calculation that under the same conditions the width or diameter of a circular aperture must be about twenty per cent greater. Here is disagreement as to the exact difference, but not as to the fact that of two apertures of the same width or diameter, one rectangular and the other round, the resolving power of the rectangular aperture is greater.

We have dwelt upon the "fundamental importance" of the contraction of the diffraction pattern with increase of aper-

* ENCYCLOPÆDIA BRITANNICA, ninth edition, Vol. XXIV, p. 430.

† *Ibid.*, Vol. XVII, p. 807. ‡ *Ibid.*

§ *The Journal of the Quekett Microscopical Club*, March, 1895, p. 28 (Nelson).

¶ *Ibid.*, p. 27 (Nelson).

ture with reference to the resolving power of the microscope, and the fact that resolving power in this instrument is proportional to its aperture. Your attention is now invited to the other optical laws quoted, though not in their order of quotation. "A double line will be about on the point of resolution when its components subtend an angle equal to that subtended by the wave length of light at a distance equal to the horizontal aperture," when aperture is rectangular and the double line is parallel with the vertical sides. Now, suppose *BD* in Fig. 2 to be the width of a rectangular aperture. By comparing the lengths of lines we have already considered in this diagram we find the arc *rp* is equal to two wave lengths of light. *AC* is equal to *BD*. Therefore, the angle *ACr* is the angle a wave length (half of the arc *rp*) subtends at a distance equal to the aperture *BD*. This angle on comparison is found to be half as great as the angle *bCc* and *fCh*. Accordingly, lines separated by an angular distance equal to half that between the lines *bC* and *cC* would be about on the point of resolution by an aperture equal in width to *BD*.

The aperture *BD** in Fig. 2 projects a central point in an object as a disc having a diameter of which the radii extend upward from *g* half way toward *f* and downward from *g* half way toward *h*. The diameter of this disc therefore subtends at the center of the objective *C*† half the angle *fCh*, or half the angle *bCc*, an angle equal to the angle *ACr*, or to the angle subtended by a wave length at a distance equal to the aperture. If a point in the object *O* at the crossing of the line *bC* be projected by the same aperture *BD*, its image will be a disc with *h* as its center, and an upper radius extending half way toward *g* where it would about meet the lower end of the diameter of the first disc. The two discs would about touch. If a point in the object *O* at the crossing of the line *cC* be projected by the same aperture, its image will be a disc with *f* as its center, and a lower radius extending half way toward *g*, where it would about meet the upper end of the

* Supposing the resolving power in each diameter of the aperture be the same as that of a rectangular aperture of equal width [also true, for simplicity, in previous use of Fig. 2].

† *C*, in Fig. 2, is both the center of the emitting surface and the center of the objective.

diameter of the first disc. These three discs on the screen about touching one another would be a picture of three points in the object about to be resolved. The centers remaining the same, lessening the diameter of the discs would result in a picture made up of three discs not touching one another; and, consequently, the corresponding three points of detail in the object would be resolved.

Now, let AE be the width of a rectangular aperature. At a distance equal to this double aperture a wave length, half of rp, would subtend the angle AEr. The angle AEr is evidently equal to half the angle ACr. Accordingly the resolving power of the double aperture should be twice as great as that of the aperture BD (equal to AC). We have already found [p. 332] this to be true: that the image discs of points have half the diameter when projected by this double aperture (as compared with image discs projected by half that aperture), and that, therefore, the resolving power is twice as great. We thus find our diagram in harmony with the law last quoted, supposing the aperture to be rectangular.

As to the difference in resolving power of a rectangular and round aperture: Lord Rayleigh, experimenting with the telescope, found a round aperture should have a diameter a little less than ten per cent greater than the width of a rectangular aperture to give the same resolution; while Sir George Airy, by calculation, found a round aperture should have a diameter a little more than twenty per cent greater than the width of a rectangular aperture to give the same resolution. To determine which result is nearer right practically in reference to resolving power in the microscope, I ruled the lines shown [not accurately reproduced] in Fig. 4; and photographed aërial images of them by means of square and round apertures. The lines were ruled on a Geneva Society dividing engine [in the physical laboratory of a kind friend, Professor E. Haanel, of Syracuse University], ruled in or cut through the opaque film of an Eastman gelatino-bromide lantern plate, exposed [to a gas jet for a few seconds], developed,

fixed, washed and dried in the usual way. The numbers indicate the distances between the lines in millimetres.

An aërial image of the lines was projected with a sub-stage condenser of $\frac{1}{4}$-inch focus. This image in the plane of the microscope stage was photographed with a Wales $1\frac{1}{2}$-inch objective, and a Powell and Lealand "compensating 20" eye-piece. Photo. 11 was taken with a round aperture of $5\frac{1}{2}$ mm.; Photo. 12, with a round aperture of 6 mm.; and Photo. 13, with a square aperture of 5 mm. (the sides being parallel with the lines). An aperture of $5\frac{1}{2}$ mm. is ten per cent greater, and an aperture of 6 mm. twenty per cent greater, than an aperture of 5 mm. Then, according to Lord Rayleigh, resolution should be nearly the same in Photo's 11 and 13—a trifle better in Photo. 11. According to Sir George Airy, it should be nearly the same in Photo's 12 and 13—a trifle better in Photo. 13. [In Photo's 11 and 13 the group of lines marked *.46* should be shown barely resolved, resolution being slightly more evident in Photo. 11. Lines marked *.42* in Photo's 11 and 13 should show no resolution, while the same lines in Photo. 12 should be shown resolved slightly better than are the lines *.46* in Photo. 13—or resolved as well as the lines *.46* in Photo. 11. These differences shown in the photomicrographs are hardly perceptible in the half-tone reproductions.] If the widths of the alternating white and gray bands [the latter of which should be black] under *1*, in Photo. 11, be critically compared with the corresponding bands in Photo's 12 and 13, it will be seen that the gray bands are broadest in Photo. 12, and of nearly the same breadths in Photo's 11 and 13. Corresponding results were got in other photomicrographic experiments.

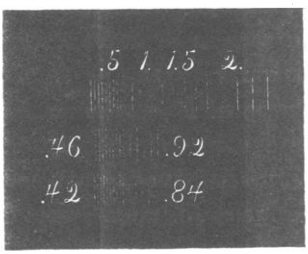


Fig. 4.

It will be noticed that the closeness of the *.46* mm. group of lines is about ten per cent greater, and that of the *.50*

mm. group about twenty per cent greater, than that of the .*42* mm. group. The closeness of the .*92* mm. group of lines is about ten per cent greater, and that of the *1*. mm. group about twenty per cent greater, than that of the .*84* mm. group. Such gradation in closeness made comparison of the results of photomicrographic experiments an easy matter. The results were found to agree with Lord Rayleigh's experiments and not with Sir George Airy's calculation.

That resolving power is "independent of focal length" when distances between details of objects are measured by the angle they subtend at the center of the projecting lens becomes evident on again studying Fig. 2. Suppose the focal length of the lens, a little less than *CO* in the diagram, to be only half as great, the screen *SN* remaining fixed. Then the object to be projected on the screen would be necessarily half as far from *C* as *O* is from *C*. The round surface *O* would require only half as great a diameter in the new position to be projected to the same pattern on the screen, by the same aperture. The lines of the diagram would have to be changed only by shifting the *O* ends of *OA*, *OB*, *OC*, *OD* and *OE* to a point half way between *O* and *C* to enable us to use Fig. 2 for demonstrating the projection of a round object having half the diameter of *O* by the lens having half the focal length. The distance between the points in the lines *bC* and *cC* touching the ends of the vertical diameter of *O* in its new position would be half that between the points in the same lines which touch the ends of the diameter in the present position of *O*. The angle subtended at *C* by the diameter of *O* in its present position is *bCc*. This is the same angle that *O* with half as great a diameter in the new position would subtend at *C*. Thus Fig. 2 would show resolving power to be "independent of focal length" when measured by the angle the distance between details subtends at the center of the projecting lens. That this optical law applies to both the telescope and the microscope [or to any other projecting lens], is what one would expect, bearing in mind the fact that a

telescope becomes a microscope by shortening sufficiently the longer of its conjugate foci, and a microscope a telescope by lengthening sufficiently the shorter of its conjugate foci.

While resolving power is independent of focal length when measured by angles subtended at the center of the projecting lens, it is not independent of focal length when measured by the actual distances between details subject to resolution. This truth, set aside early in our study to simplify discussion, may here be considered. We have just seen that *O* in Fig. 2 would require only half as great a diameter to be projected to the same pattern on the screen *SN*, if the lens had half as great a focal length. Therefore, what was said in a preceding paragraph [page 340] regarding the projection of three points of detail in the object *O* would be true of three points of detail half as far apart in the smaller object, half way between *O* and *C*, projected on the screen *SN* by a lens of half as great a focal length. Then, in turn, neglecting the angles subtended at the center of the projecting lens by distances between the details of an object, we find that resolving power relating to actual distances in an object projected by the same aperture varies inversely with focal length. With the same aperture, a $\frac{1}{2}$-inch objective would resolve a series of lines half as far apart as would a 1-inch objective.

Photo. 15, of an aërial image of the ruling shown in Fig. 4, was taken with a $\frac{1}{2}$-inch objective having an aperture of 2 mm.; and Photo. 16 of the same aërial image, with a 1-inch objective having an aperture of 2 mm. Different eye-pieces were used so as to get nearly the same amplification. The relative closeness of the lines is shown in Fig. 4. Compare the lines *1* unit apart in Photo. 15 with those *2* units apart in Photo. 16. Compare these groups with the others in the two Photo's as to the comparative width of lines and the intervening spaces. Of all the lines in Photo. 16 those *2* units apart are, lines and interspaces, most like those *1* unit apart in Photo. 15 (the interspaces between the lines *1.5* and *2* units

apart in Photo. 15 being comparatively too wide). There is additional evidence in the photomicrographs [not seen in the half-tone reproductions], showing that with the same aperture resolving power varies inversely with focal length when distances between the details are subject to linear instead of angular measurement.

The two expressions regarding the relations of resolving power and focal length are, however, quite in harmony. While resolving power measured by angles, at the center of the projecting lens, subtended by details in an object is independent of focal length, a shorter focal length (for instance) necessitates a shorter distance between the object and the objective. This results, on the one hand, in the same details subtending at the center of the projecting lens a proportionately greater angle; or, on the other hand, proportionately less separated details subtending the same angle. In so far as the details subtending the same angle are less separated as a result of shorter focal length causing the object to approach the objective, resolving power relating to actual distances in the object is proportionately increased.

We have seen that the resolving power relating to actual distances between the details of an object [and this is the common meaning of resolving power in the microscope] is, first, inversely proportional to the angle (at the center of the projecting lens) a wave length of light subtends at a distance equal to the aperture. Secondly, it is inversely proportional to the focal length of the projecting lens. This means (referring again to Fig. 2) that by decreasing the angle ACr, or by causing the object O to approach the lens, resolving power is increased. Practically, there are limits to these two operations, and hence a limit to resolving power.

Let us see how these limits apply. It is practicable to cause an object to so far approach a lens that the object all but touches the glass, while the objective takes in from the object a cone of light having an angle of very nearly 180°. In such an instance a limit to resolving power is practically reached. The object could not be nearer the objective;

and greater aperture could not be utilized, because the cone of light from the object reaches approximately the highest possible angular limit. But such a limit reached in the case of a dry objective used with white light can be passed, and is passed, in the case of an immersion objective, or a dry objective used with light of shorter wave length. Consider for a moment the use of monochromatic blue light of shorter wave length: As with such light the arc rp in Fig. 2 would be shorter, and the angle ACr proportionately less, resolving power—varying inversely with this angle—would be greater. Increase of resolving power with the use of blue light is recognized in practice, particularly in photomicrography. With the use of light of shorter wave length the angle ACr is lessened by shortening the subtending arc rA. With the use of immersion objectives this angle is lessened by increasing AC, or the diameter of the emitting surface BD.

In Fig. 5, let O be a point in an object and L the front lens of a dry objective. Let aOb be a cone of light leaving O and entering the lens L. Suppose the ray Oa to be refracted by the surface vw so as to reach the surface xz at c and the ray Ob so as to reach the surface xz at d. The diameter of the emitting surface is then cd. Now, suppose O and the cone aOb to be in a fluid having the same index of refraction as the glass of the lens L. Then the rays Oa and Ob would suffer no change in direction on entering the lens, Oa reaching the surface xz at m and Ob the same surface at n. The diameter of the emitting surface would now be increased by the difference between cd and mn. The greater the index of refraction of the lens L the greater would be the increase of aperture by homogeneous immersion. Suppose the lens L to have a higher index of refraction. Then a cone of light in air of greater angle, vOw, might after refraction by the surface vw be emitted by the surface d. Now, suppose O and the cone of light vOw be in a fluid

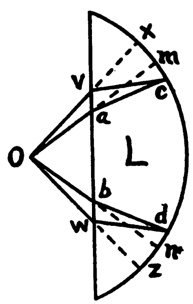


Fig. 5.

having the same index of refraction as the glass of the lens *L*, with its higher refracting power. The rays *Ov* and *Ow* would suffer no change in direction on entering the lens, *Ov* reaching the surface *xz* at *x* and *Ow* the same surface at *z*. The diameter of the emitting surface [or effective aperture] would now be increased by the difference between *cd* and *xz*.

A few lenses have been made of glass of exceptionally high refractive index, requiring a special homogeneous immersion sub-stage condenser, stage slips and covers made of special glass, as well as an immersion fluid of correspondingly high refractive index. Such lenses and necessary accessories made by Zeiss, of Jena, are very costly and very limited in usefulness, while the gain in aperture is only about 60 per cent (over dry apertures) as compared with the gain of about 50 per cent in ordinary homogeneous immersion objectives.

The estimating of resolving power as to actual distances in a microscopic object is a complicated matter ; for, in addition to aperture as a factor, we have found a consideration of the effects of focus and immersion fluid must be included. The meeting of this difficulty by Professor Abbe conferred a boon upon microscopists. By means of Professor Abbe's "numerical aperture"* [expressed by N. A.] it becomes a comparatively easy matter to ascertain the resolving power of microscope objectives. Numerical aperture tables, exhibiting the theoretical limit of resolving power under different conditions of wave length, immersion fluid and aperture, are now widely accessible, for instance in the Dallinger-Carpenter edition of *The Microscope and Its Revelations*, p. 84. These tables, however, are based upon the Abbe theory and, therefore, exhibit too high a limit—that is under conditions suitable for best microscopic vision [a matter to be considered later].

A study of the effects of projection by zonular apertures is not only interesting but of practical importance ; because

* For an explanation of "numerical aperture" the reader is referred to Chapter II in the Dallinger-Carpenter edition of THE MICROSCOPE AND ITS REVELATIONS. In this paper aperture means opening, or extent of opening measured by its diameter or breadth.

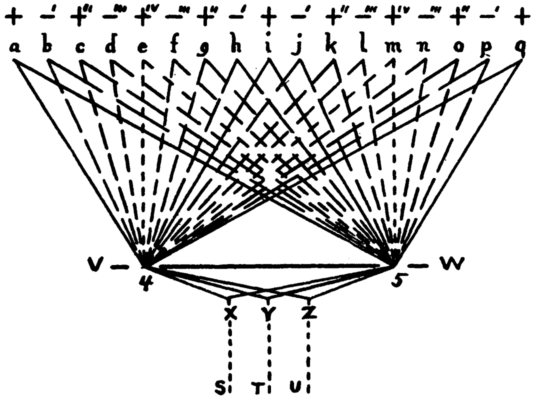


Fig. 6.

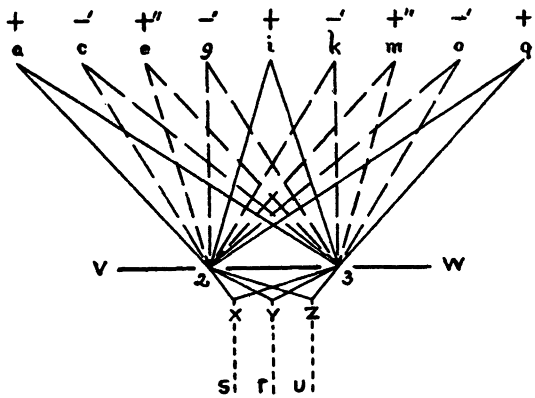


Fig. 7.

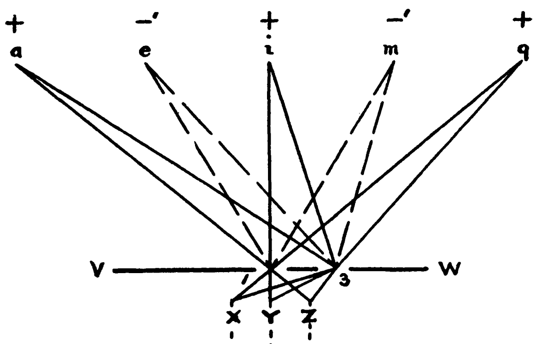
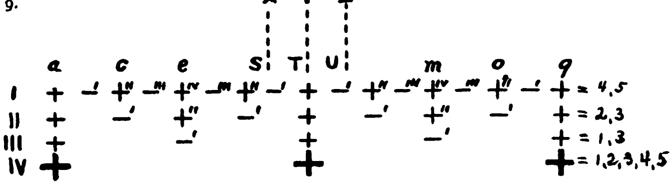


Fig. 8.

Fig. 9.



both objectives and sub-stage condensers are commonly not aplanatic, different zones having different foci. The effect of projection by one zone of an objective may be seen with one focusing and that of projection by a different zone with another focusing. The importance of having but one focus for all zones of the objective [and of the sub-stage condenser which by having different foci for different zones sends primary rays, at any one focusing, only to the one zone in the objective corresponding to that which is at the time operative in the sub-stage condenser] becomes evident on studying Fig's 6, 7, 8 and 9, and corresponding photo-micrographs.

In Fig's 6, 7 and 8, let *VW* be a section of a diaphragm in contact with the emitting surface of a projecting lens. Let *X*, *Y* and *Z* be three self-luminous points (or points illuminated by a full cone of sub-stage light) in one of the conjugate foci of the projecting lens, and *a*, *i* and *q* primary ray images of *X*, *Y* and *Z* in the other of the conjugate foci. In Fig. 6, the diaphragm *VW* has two slots of which *4* and *5* are transverse sections; in Fig. 7, two slots of which *2* and *3* are transverse sections; and in Fig. 8, two slots of which *1* and *3* are transverse sections. The distance between the slots *4* and *5* is twice as great as the distance between the slots *2* and *3*, and four times as great as the distance between the slots *1* and *3*. The distance between *X* and *Y* (and *Y* and *Z*) is supposed to subtend a very small angle at the center of the projecting lens. [The distance is thousands of times the actual distance; and, as in previous diagrams, the wave length used is thousands of times too great. This is necessary to enable us to deal with measurements great enough to be useful.] Each slot is supposed to uncover one point of emission. Primary rays are indicated by unbroken lines, and secondary rays by broken lines. The broken character of the lines in any one of the diagrams increases with the obliquity of the secondary ray to its primary ray. For instance, *4k* and *4m* in Fig. 6 are secondary rays from the primary ray *4i*. The line *4m* is more oblique to *4i* than *4k* is to *4i*; and *4m* is more broken than *4k*.

In Fig. 8, each slot allows one primary ray to reach each of the images *a*, *i* and *q*. The images *a*, *i* and *q* are not as perfect as the diagram indicates; for there would be to the right and to the left of the points *a*, *i* and *q* such spreading of light as we have before fully considered. At each slot in Fig. 8 a primary ray gives rise to secondary rays. One secondary ray from each slot reaches *e*, and one from each slot *m*. [In this, and Fig's 6 and 7, secondary rays to the left of *a* and to the right of *q* are not considered. They would take paths symmetrical with the secondary rays in paths to the right of *a* and to the left of *q*, respectively.] The secondary rays *1e* and *3e* differ in length by half a wave length, are opposed in phase and interfere; and *e* is dark. Correspondingly, *m* is dark. Over *a*, *e*, *i*, *m* and *q* in Fig. 8 are plus and minus signs indicating illumination and absence of illumination, corresponding to the same signs in Fig's 1 and 2.

After studying Fig's 1, 2 and 8, it is easily understood how the emitting points *2* and *3*, in Fig. 7 [twice as far apart as *1* and *3* in Fig. 8] project images of *X*, *Y* and *Z* at *a*, *i* and *q*, with diffraction patterns extending to the right and left of *a*, *i* and *q*, respectively, equal to half those projected under the conditions of Fig. 8. Thus *c* is dark, the result of union of secondary rays opposed in phase; while *e* is illuminated, the result of the union of secondary rays agreeing in phase. In a similar way, *g*, *k*, *m* and *o*, with their plus and minus signs, are accounted for.

In Fig. 6, the diffraction pattern, indicated by the plus and minus signs above the letters *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i*, *j*, *k*, *l*, *m*, *n*, *o*, *p* and *q* and projected by emitting points *4* and *5* [twice as far apart as the emitting points *2* and *3* in Fig. 7], is also easily accounted for in a manner similar to that in which we have accounted for previous diffraction patterns.

Now let us suppose all the emitting points, *4*, *2*, *1*, *3* and *5*, in Fig's 6, 7 and 8, to be simultaneously effective. The patterns of Fig's 6, 7 and 8 would unite. The result of the union is imperfectly expressed in Fig. 9, where the plus and minus values of the three diffraction patterns are brought

together and added, as it were. Line *I* stands for the pattern of emitting points *4* and *5* in Fig. 6; line *II*, for the pattern of emitting points *2* and *3* in Fig. 7; line *III*, for the pattern of emitting points *1* and *3* in Fig. 8; and line *IV*, for the picture which union of the three patterns tends to produce (or the image of *X*, *Y* and *Z* the emitting points *4*, *2*, *1*, *3* and *5*, simultaneously effective, tend to project). The plus signs corresponding with the primary ray image *a*, *i* and *q*, reinforce one another, while the plus and minus signs corresponding to secondary ray interference phenomena tend to nullify one another. Fig. 9 [incomplete and not accurate*] serves to express graphically the general truth that multiplying the number of effective slots, or zones, intensifies the primary ray image, while diffraction phenomena tend to disappear.

Of the diffraction patterns projected in Fig's 6, 7 and 8, that of Fig. 8 [line *III* in Fig. 9] is nearest like that which the five slots jointly tend to project. But the latter is much more intense, and the spreading of light to the right and left of *a*, *i* and *q* is only one-fourth that in the former. The latter approaches four times as near being a dioptric image.

If *X*, *Y* and *Z* were sections of self-luminous lines (parallel with slots in the diaphragm *V W*), *a*, *i* and *q* would be sections of primary ray image bands. In addition to the bands *a*, *i* and *q* in Fig. 7 two diffraction lines, *e* and *m*, would appear. In Fig. 6 would appear six diffraction lines, *c*, *e*, *g*, *k*, *m* and *o*. The actual number of lines to the inch in the object would appear to be doubled, and half as far

* Some points not shown, or not shown accurately, in Fig. 9: The primary ray image projected by five slots has a brightness greater than that projected by any two of the same slots in the ratio of five to two [due to the actual increased amount of light leaving the lens and reaching the image]. Again, when the five slots are simultaneously effective the diffraction phenomena of line *I*, Fig. 9, can occur but once, because of the five slots only two are sufficiently far apart to project such a pattern; while the diffraction phenomena of lines *II* and *III* are intensified by more than one inter-pairing of the five slots. Therefore the latter patterns have more light, respectively, than the former. On the other hand, the diffraction phenomena of line *I* are contracted laterally [as by a wide aperture] so as to be twice as intense as the diffraction phenomena of line *II*, and four times as intense as those of line *III*. The intense thin diffraction phenomena of line *I* are likely to show under certain conditions as residua of opposing diffraction effects, even when the slots are made a continuous aperture by cutting away the intervening bars—as is shown at *c*, *e* and *g* (corresponding to the same letters in Fig. 6) in Photo. 29. Moreover, to Fig. 9 should be added another diffraction pattern projected as the effect of the inter-pairing of slots three times as far apart as slots *1* and *3*, which would present two equidistant plus phenomena between *i* and *q*, instead of the three shown in Fig. 6.

apart, in Fig. 7; and quadrupled, and one quarter as far apart, in Fig. 6. If *V W* were a section of every diameter of the diaphragm, the slots *2* and *3*, and *4* and *5*, would become annular or zonular. Then *i* in Fig. 7 would be surrounded by a diffraction ring of light of which *em* would be the diameter. At *e* this ring would touch a similar ring surrounding *a*, and at *m* another similar ring surrounding *q* [dots and rings in the middle upper portion of Photo 6]. In Fig. 6, *i* would be surrounded by two rings having, respectively, the diameters *gk* and *em*. Two similar rings would surround *a* and *q*. The larger ring about *i* would touch the larger ring about *a* at *e*, and the larger ring about *q* at *m*. If *X*, *Y* and *Z* were sections of self-luminous lines, each point in a line would be projected as a disc with concentric rings. The discs would overlap in one direction to form a band, while the rings would cut one another so as to emphasize tangential lines parallel to the bands [see bands, semi-rings at their ends, and parallel tangential lines between them, in several portions of Photo. 6].

Experiment 5: The apparatus and its arrangement were the same as in Experiment 3, with two exceptions. The exceptions were: first, for the two pinholes were substituted three pinholes (in line, about $\frac{1}{2}$ mm. in diameter and 2 mm. apart from center to center) and, near the pinholes, a group of three parallel slits (about $\frac{1}{2}$ mm. wide and 2 mm. apart middle to middle); and, secondly, for the diaphragms with a single opening used at the back of the objective were substituted diaphragms with two slots. Images of the pinholes and slits were projected by the sub-stage condenser, with full aperture, in the plane of the microscope stage, where they became essentially self-luminous aërial dots and lines. The dots corresponded with the self-luminous points *X*, *Y* and *Z* in Fig's 6, 7 and 8. Diaphragms were made to correspond with *VW* in the diagrams. The distance corresponding to *1-3* in Fig. 8 was 1 mm.; the distance *2-3* in Fig. 7, 2 mm.; and the distance *4-5* in Fig. 6, 4 mm. A fourth diaphragm had five slots, 1 mm. apart, which would uncover five points of

emission corresponding with *4*, *2*, *1*, *3* and *5* in the diagrams. The slots were equal in length.

The picture of the dots and lines seen through the microscope, with the full aperture, is shown in Photo. 1 as *a*, *i* and *q*, and at *a*, *i* and *q* repeated at an angle of 90°. [When taking the photomicrographs: an arc light and bull's-eye lens were substituted for the lamp; between the pinholes and slits, in a black card, and the sub-stage condenser was a blue light filter; the microscope tube was horizontal and the mirror was not used.] The full aperture image shown in Photo. 1 is nearest like the original object. Projected with five isolated slots, the image shown in Photo. 5 differs more; while with two isolated slots, the picture shown in Photo's 3 and 4 (corresponding with Fig's 7 and 6, respectively) differs remarkably from the original object.

Photo's 1, 2, 3, 4 and 5 present a pictorial warning against the use of isolated zones [each behaving like two slots, in every diameter], often utilized unwittingly by the practical worker [a result of imperfect correction of spherical aberration in either the objective or sub-stage condenser]; for if such simple details as we have studied are changed in appearance so remarkably, imagine what must be the corresponding changes presented to an observer when studying complicated structures!

In taking Photo. 1, an aperture of 10 mm. was used. In taking Photo. 29, a continuous aperture of about 4 mm. (just sufficiently wide to take in the emitting points *4* and *5* of Fig. 6) was used. In the latter a faint diffraction pattern, corresponding to that in Fig. 6, is seen partly at *c*, *e* and *g*, and partly between *i* and *q*; while in Photo. 1 such diffraction phenomena are absent. Great intensity of the light used, or long exposure of the photographic plate, may favor the projection of such a diffraction pattern as that seen in Photo. 29 [residual—see footnote, p. 351].

This matter of intensity of light in relation to aperture is worthy of little attention. If late in the day a microscope be arranged so as to resolve an object, with deepening twilight the closest and presently the less close details disappear.

Similar phenomena are met with in ordinary work as the result of poor illumination. On the other hand, "drowning" with an excess of light [sometimes softened by using a blue glass filter] is an effect commonly seen.

There is an intermediate intensity best for microscopic vision. Bearing this in mind, if the intensity of the light in primary ray image bands were to measure twenty units and that of certain parallel diffraction lines one unit, the latter being barely visible, illumination could be lessened ninety-five per cent and still be sufficient to enable an observer to see the primary ray image bands, while the diffraction lines would be far too faint to be seen. On the other hand, if the diffraction lines of one unit intensity [barely visible] were to be illuminated twenty times as brilliantly to give them proper visual intensity, the primary ray image bands would be twenty times too intense and might suffer from "drowning" effects. The less the difference in intensity between the primary ray image bands and diffraction lines, the more likely are diffraction phenomena to obtrude themselves in a picture when the primary ray image has proper visual intensity. The use of pairs of slots or isolated zones, as compared with the use of continuous apertures, lessens this difference in intensity and favors the obtrusion of diffraction phenomena [compare *c*, *e* and *g* in Photo's 4 and 29, the former taken with a pair of slots and the latter with a continuous aperture].

Experiment 6: The general arrangement of the apparatus and the aërial object remained the same as in the last experiment. But instead of rectangular slots, annular or zonular slots were used at the back of the objective.

When an annular slot exposed emitting points in a circle 2 mm. in diameter, the image discs were surrounded by rings of light having radii corresponding to *ae*, *ie*, *im* and *qm* in Fig. 7, and shown in the middle upper portion of Photo. 6. When the annular slot exposed emitting points in a circle 4 mm. in diameter, the discs were surrounded by closer rings, the radii of the smallest corresponding with *ac*, *ig*, *ik* and

qo, and the radii of the next larger with *ae*, *ie*, *im* and *qm*, in Fig. 6, and imperfectly shown in the middle upper portion of Photo. 7. The tangential union of diffraction rings between the bands [overlapping discs] results in intense lines with which, by comparison, the semi-rings at the ends of the bands appear faint [better seen in Photo. 6]. Portions of each ring are lost by interference in the intervals between the bands and the tangential lines. The annular slot or zone 2 mm. in diameter used in taking Photo. 6 was complete [a coverglass elsewhere opaque with India ink], while that 4 mm. in diameter used in taking Photo. 7 was broken [a stencil cut out of black paper]. Of the diffraction rings in the photomicrographs, those projected with the former zone are continuous lines, while those projected with the latter are broken —showing how sensitive the diffraction pattern is to modifications of aperture. The difference is imperfectly seen in the half-tone reproductions.

In considering the photomicrographs up to this point we have, for the most part, dealt with intensity in the one diameter corresponding to the widths of the lines photographed. Because the slots used had the same length, the intensity depending upon such lengths remained the same notwithstanding the changes experimentally made in the other diameter. We shall now see that this is an imperfect view of the matter. We have dwelt upon the facts that the diffraction pattern is contracted with every increase in diameter and that this is true in all diameters. We know that with circular lenses an area of diffracted light [a portion of a diffraction ring or line] varies in extent inversely with the square of its diameter or aperture. Therefore the light intensity of such an area would vary directly with the square of the aperture, provided the amount of light transmitted by the objective were to remain the same. But the amount of light transmitted by the objective increases with the square of its aperture. This means that, independently of the contraction of the diffraction pattern, an area of light in the projected image varies in intensity with the square of the

aperture of the lens. Increase of aperture, then, adds to the intensity of the diffraction pattern in two ways. The increase of intensity gained in one way must be multiplied by that gained in the other, to get the total increase. The result may be expressed thus: *The intensity of the diffraction pattern varies with the square of the square of the aperture.*

Experiment 7: The apparatus and conditions remained the same as in the last experiment, excepting: for the object was substituted an aërial image of a tiny pinhole; and for two or more slots back of the objective single slots were substituted.

Photo. 8 is a double photomicrograph of the aërial object. *E* had the first exposure of fifteen seconds, with a slot 2 mm. wide. The aërial object was shifted very slightly to the right. *F* had an exposure of 1,215 seconds, with a slot 1 mm. wide. Exposed on one plate, the two images had the same treatment, in development, fixing and washing, and, therefore, could properly be compared as to their intensities. [The triple broadening of the disc, by the lesser aperture, at once attracts attention.] The exposures were so timed as to show the first diffraction ring in each case, provided the expression at the close of the preceding paragraph be true. [The square of the square of the aperture 1 is 1. The square of the square of the aperture 3 is (3 x 3) x (3 x 3), or 81. The exposures were as 1 to 81.] In the negative it was impossible to say that the two rings differed in intensity. Unfortunately the half-tone process has failed to reproduce the rings.

As a dioptric image would not be contracted by increase of aperture, its intensity would increase with simply the square of the aperture. Broad image areas, except at their boundaries, also increase in intensity with simply the square of the aperture. As such areas are made up of overlapping images of points, of which the object may be considered an aggregation, any increase of intensity due to contraction of the overlapping discs within the boundary bands is balanced by a decrease due to less overlapping.

That boundary bands of image areas and images of isolated lines are affected alike by aperture is shown in Photo's 25 and 26. They were taken under the same conditions present in taking Photo. 1, excepting that, instead of full aperture, a rectangular slot measuring 2 mm. by 6 mm. was used back of the objective. In taking Photo. 25, the length of the slot was horizontal; in taking Photo. 26, vertical. Careful measurements show that there is broadening of the areas *A* and *B* with the lesser aperture in each case, and that the broadening is such as can be accounted for by the widening of the boundary bands. The narrower the area, the more important to the total breadth of the area is the broadening of the boundary bands. With small apertures the visual pictures of finest particles and lines consist chiefly of diffraction spreading. Such diffraction spreading not only contracts with increasing aperture, but gains in intensity rapidly [with the square of the square of the aperture]. Thus, increasing aperture narrows and intensifies most noticeably the picture of finest details. In a similar way, increasing aperture causes diffraction spreading of boundaries of areas to contract and to approach in intensity that of the area, as the intensity of the former increases with the square of the aperture, while that of the latter increases with the square of the square of the aperture.

Does not a study of Photo's 25 and 26 suggest a field for thought regarding micrometry? For instance, recall the disagreement of numerous careful workers who have measured blood corpuscles. Has one such worker recorded any consideration of the aperture used? Will not such a record come to be regarded as an essential part of critical micrometry?* While the percentage of error may not be great in

* A column might be added to numerical aperture tables to show what amount should be deducted for each N. A., with, for instance, an amplification of one hundred diameters. Astronomically, the diameter of, for instance, the sun's true image is increased at each end by half the diameter of a star disc; and, therefore, a measurement of the sun's diameter should be corrected for the aperture of the telescope used by subtracting the diameter of a star disc measured, if measurable, by means of the same instrument, or one of the same aperture. The uncertain [fading, not sharp] character of the visual boundaries of projected images also contributes to the uncertainty of micrometry.

the measurement of broad areas, it is likely to increase rapidly as comparatively minuter objects and details are measured.

That a boundary of an area and an isolated line are affected alike by aperture is again shown in Photo's 23 and 24. The former, of an aërial image, was taken under the same conditions under which Photo. 1 was taken. The latter was taken under the same conditions under which Photo. 2 was taken. The right boundary is continuous with an isolated line, both of which have been affected alike in Photo. 24 by aperture. A study of the boundaries of the broader areas and of the dots and lines in Photo's 2, 3, 4 and 5, as compared with the same in Photo. 1, furnishes more evidence to the same effect.

A boundary band is not exclusively affected by aperture. Its next overlapping neighbor, and each succeeding band in the light area, has the same diffraction pattern. The lines of the diffraction patterns touch each other and produce the step-like areas of graded tints outside the boundaries of the light areas, seen in Photo. 5 [poorly shown in the reproduction]. Residua of these tints are also seen at the horizontal boundaries of *A* in Photo. 25 and at the vertical boundaries of *A* in Photo. 26.

The formation of these tints can be explained by means of Fig. 7. Suppose *YT* and *ZU* to be lines in a light area extending from *YT* to the right far beyond *ZU*, and *XS* to be absent. The area may be regarded as made up of lines parallel to *YT* touching one another. Images of the lines would be projected as overlapping bands extending from *i* to the left beyond *a*. Each successive band in the image to the left of *i* would, if isolated, have a diffraction pattern indicated by *k*, *m*, *o* and *q*. Thus *k*, *m*, *o* and *q* would be repeated for each successive band. To the right of the right boundary of the area projected to the left of *i*, the repetitions of *q* just touching one another would extend from *q* to *i*, and the repetitions of *m* from *m* to *i*. The tint between *q* and *m* would be, for the most part, an intensity of light resulting from the simultaneous effect [compounded wave motion] of the repetitions of plus *q* and minus *o*. The tint between *m* and *i*

would be, for the most part, an intensity of light resulting from the simultaneous effect of the repetitions of plus *q*, minus *o*, plus *m* and minus *k*. Because the phenomena between *m* and *i* are more intense than those between *q* and *m*, in inverse relation to their obliquity, and because the phenomena between *q* and *m* would be repeated and compounded with the phenomena between *m* and *i* in the overlapping of diffraction patterns [the intensity of light between *q* and *i* being only slightly increased by the overlapping of fainter portions of the pattern which are not indicated in the figure but lie to the right of *q*], the tint between *q* and *m* would be considerably less bright than the tint between *m* and *i* [and the next tint to the right of *q* would be proportionately faint].

If the projected image of *YT* were fully shown by the pattern extending from *a* to *q*, repetitions of the pattern to the left of *a* would mutually and completely overlap one another, and brightness would there be uniform. Between *a* and *e*, within the image of the bright area, overlapping would be incomplete ; and between *e* and *i*, also within the image of the bright area, less nearly complete. As a result : a tint, less bright than that of the image area to the left of *a*, would be seen between *a* and *e* ; and a tint of still less brightness, between *e* and *i*. These tints within the image of the bright area would be more intense than the tints outside the image area, where overlapping becomes gradually less and finally does not occur.

We are now prepared to reverse our explanation and show how such boundary tints disappear when isolated slots are replaced by a continuous aperture. Because boundary tints depend upon the presence of diffraction patterns, the former disappear with the disappearance of the latter. The right boundary line of one tint is *m* [a false image corresponding to nothing in the object]. The repetition of this diffraction line between *m* and *i* essentially constitutes the tint in that situation. Then, in order to eliminate the tint between *m* and *i* from the picture, it is only necessary to eliminate the line *m* from the diffraction pattern.

Suppose every point of emission between *2* and *3* in Fig. 7 to be uncovered, the point *1* [see Fig. 8] would emit light along a path to *m* half a wave length longer than the path *3m* [the path *2m* being in the diagram a wave length longer than *3m*]. A ray from each succeeding point between *2* and *1* would take a path to *m* half a wave longer than a ray from each succeeding corresponding point between *1* and *3* would take to *m*. The rays in the paths between *2m* and *1m* being longer than the rays between *1m* and *3m* by half a wave length, pair by pair, would be opposed in phase. The false line *m* would be replaced by darkness. Therefore darkness would extend from half way between *qo* on the right to half way between *ki* on the left. *SX* not being present *q* would be a first diffraction line, produced by the union of secondary rays in paths between *2q* and *1q* with those in paths between *1q* and *3q*—the former being, pair by pair, a wave length longer than the latter. If *SX* were present, the primary ray image at *q* would be intensified by the union with it of the same secondary rays. We thus see that all emitting points in an aperture are important. By using them, as compared with the use of isolated slots or zones, the picture improves by eliminating from it false diffraction appearances.

Photo. 30 is of aërial lines taken with the same general arrangement of apparatus. But for the sub-stage condenser another—a Powell and Lealand achromatic of $\frac{1}{5}$-inch focus—was substituted; and for the objective, a Powell and Lealand $\frac{1}{2}$-inch. At the back of the objective was placed a diaphragm having three slots corresponding to *4*, *1* and *5* in Fig's 6 and 8, or the equivalent of a pair of slots corresponding in distance apart to *4* and *5* in Fig. 6, or a pair and a half of slots corresponding in distance apart to *2* and *3* in Fig. *7*. The effect of such a combination is indicated in a general way by an imaginary union of the lines *I* and *II* of Fig. 9, and is seen in Photo. 30 at *a*, *e*, *i*, *m* and *q*. Plus *e* and plus *m* of the first line unite with plus *e* and plus *m* of the second, in Fig. 9, to form the bright lines *e* and *m* of Photo. 30. Owing to the contraction in the breadth of the plus effect represented

by *c*, *g*, *k* and *o* in the first line, the effect is 100 per cent more intense than the minus effect of *c*, *g*, *k* and *o* in the second line when both are projected by two equal slots. But the latter effect now projected by three slots has its intensity increased by 50 per cent. This intensity, however, is still 50 per cent less than that of the former, projected by only two slots [only two of the three being far enough apart]. Residua of plus effect corresponding to *e*, *g*, *k* and *o* in the first line give the corresponding faint lines seen in Photo. 30 (but indistinctly shown in the half-tone reproduction between the lines *a* and *e*, *e* and *i*, *i* and *m*, and *m* and *q*). The same pattern is better shown in the reproduction of Photo. 31.

The different effects studied by means of Fig's 6, 7, 8 and 9, and shown in Photo's 2, 3, 4 and 5, depend upon varying the distances between the slots, the distance between *X* and *Y*, or *Y* and *Z*, remaining the same. The same effects may be got in the inverse way by varying the distances between the lines or details of an object, the distance between a given pair of slots remaining the same, as may be seen in Photo. 4 in which projection by two slots, corresponding with *4* and *5* in Fig. 6, causes the bands *a*, *i* and *q* of a primary ray image to appear four times too numerous to the inch. To the right of these bands is projected by the same slots a picture of lines half as far apart as the lines *a*, *i* and *q* [compare the two series of lines as shown in Photo. 1], which presents the same appearance an image of *a*, *i* and *q* would present if projected by two slots corresponding with *2* and *3* in Fig. 7.

In Photo's 29 and 30 are seen somewhat different effects due to varying distances between lines, the diaphragm back of the objective remaining the same. The image to the right of *a* in Photo. 30 was projected, as we have seen previously, by three slots corresponding to *4*, *1* and *5* in Fig's 6 and 8. The pattern just to the left of *a* is that which would be projected by slots corresponding to *4* and *3*, or *2* and *5*, in Fig's 6 and 7. Compare this with the corresponding, but residual, pattern under *1.5* in Photo. 29. The pattern *c*, *e* and *g* in Photo. 29 is similar to that which would be pro-

jected by slots *4* and *5* in Fig. 6, or that shown in Photo. 4. The pattern under *1* in Photo. 29, with one faint diffraction line between the bands, is similar to that which would be projected by slots *2* and *3* in Fig. 7, or that shown in Photo. 3 at *e*, *m*, *a*, *i* and *q*. The pattern to the left of the last, barely showing resolution, is similar to that which would be projected by slots *1* and *3* in Fig. 8, or that shown in Photo. 2 at *a*, *i* and *q*.

Although Photo. 29 shows patterns similar to those which a pair of slots would project of lines different distances apart, the picture was in reality taken by a continuous aperture—a slot just wide enough to barely resolve the closest series of lines shown as image bands in the first position [above and to the left in Photo. 29], twice wide enough to resolve the series shown in the second position, three times wide enough to resolve the series shown in the third position, and four times wide enough to resolve the series shown in the fourth position. Between *a*, *i* and *q* in the fourth position are seen the residua already mentioned corresponding to *c*, *e* and *g* of Fig. 6 or of Photo. 4. Between the image bands in the third position are seen the residua of the corresponding broader lines of Photo. 30. Between the image bands in the second position are seen the residua of lines corresponding to *e* and *m* of Fig. 7 or of Photo. 3.

Why do residua of light corresponding to diffraction lines show in Photo. 29 and not in Photo 1? Partly because Photo. 29 had a relatively long exposure, Photo. 1 having only sufficient exposure to enable the primary ray image to properly affect the sensitized plate. The sensitized plate saw, as it were, the projected image in Photo. 1 with a proper intensity of light. In Photo. 29 the intensity was unnecessarily great, but not great enough to give the "drowning" effect seen in Photo's 6 and 7. If Photo. 1 had received relatively the same exposure given Photo. 29, the greater aperture effective in taking the former would have contracted the pattern so that the diffraction lines could not have been seen as separate from the primary ray bands without the

aid of a pocket lens. This was found to be true by actual test.

To what is the "drowning" effect seen in Photo's 6 and 7 due? In the beginning of this paper the inverse relation of the sizes of image discs, due to uncorrected spherical and chromatic aberrations on the one hand and to aperture on the other, was noted. In so-called achromatic objectives these aberrations are at best incompletely corrected. In apochromatics they are not perfectly eliminated. Residual aberrations increase with aperture. Aberrant light, if isolated, would give rise to aperture diffraction phenomena. It would be reflected in ways similar to those we have studied in the instance of primary ray light. The rays of each color would project patterns peculiar to and depending upon their respective wave lengths. Not isolated, aberrant rays unite at lens surfaces with other rays leaving the same points, to be compounded in resultant wave motion. Thus, aberrant light complicates wave motion. This results in a failure on the part of the proper proportion of rays to fully nullify one another and in residual diffraction phenomena spreading out in the picture beyond the boundaries and in the interstices of the primary ray image. Sufficiently brilliant illumination gives such residual light a visible intensity. Aberrant reflections at numerous lens surfaces, any method of illuminating an object which brings a zone of aperture into separate use, and certain accidents of construction in the objective or substage condenser, result in an overspreading of light the intensity of which increases with the brilliancy of illumination. As a result of one or more of these several causes the intervening spaces between the details of microscopic images come to be occupied by more or less light. Under conditions of ordinary usage it is not particularly noticeable. But by excessive brilliancy of illumination or by excessive aperture (relatively to poor correction of aberrations) increasing residual aberrations, or by both, it may become visible or with prolonged exposure affect the photographic plate. This is evident in the results got in several negatives which were

secured in attempting to photograph diffraction rings, especially those of second and third intensity shown in Photo. 7. [Such an explanation of "drowning" effect suggests an unrecognized factor—so far as the writer knows—in the production of photographers' "halation" which has been thought to be due wholly to reflection from the back surface of the negative plate or to the lateral spreading of chemical action in the film, or both. On seeing the "drowning" effects in Photo's 6 and 7, the photographer would say they were due to "halation."]

It was found that a thin cell with plate glass sides containing a deep blue solution of ammonio-sulphate of copper placed anywhere back of the sub-stage condenser rids a visual picture of much of the "drowning" effect by reducing the intensity of illumination and eliminating some of the phenomena due to other than blue wave lengths. Even with the blue cell, which is less monochromatic with the increasing intensity of the light it filters, long exposure gave the results seen in Photo's 6 and 7 [on double-coated "non-halation" plates which gave better results than the ordinary plates also tried].

Experiment 8: The apparatus was arranged as it was when Photo's 1 to 6 were taken. But instead of an aërial image of bright lines and areas an aërial image of dark lines and areas was photographed. The results in Photo's 19, 20, 21 and 22 correspond with those in Photo's 1, 2, 3 and 5, respectively. Broadening of black bands in the image by narrow aperture is seen in Photo. 20; the production of false diffraction lines (*e* and *m*), in Photo. 21; and the effect of using five slots instead of two, in Photo. 22. In this experiment the results are apparently analogous to those obtained with bright lines as an object. But the black details are fainter than the bright details in the corresponding photomicrographs. This was more marked before Photo's 19, 20, 21 and 22 were intensified. [These alone of the photomicrographs submitted were intensified to aid the half-tone plate makers.] On comparing broad areas the positive or bright effects tend to

overbalance the negative effects. Bright areas in a dark field appear too broad, due to spreading of light into the dark field. Dark areas in a bright field appear too narrow, due to spreading of light from the field into the dark areas. This is seen in comparing *A* and *A* in Photo's 1 and 2 with *A* and *A* in Photo's 19 and 20.

Photo. 23 is of an aërial image taken under the same conditions under which Photo's 1 and 19 were taken ; and Photo. 24, of the same aërial image taken under the conditions under which Photo's 2 and 20 were taken. In the upper part of the picture is seen a negative band (*a*) in a bright area and also a bright band (*b*) in a negative area. In Photo. 24 the false diffraction line duplicates of these two bands are formed as though the fellow band and duplicates were absent. The diffraction duplicates and the primary ray image bands vie with each other for the same places in the picture, with a result to the advantage of the bright line at every place. [The half-tone reproductions show the negative effects imperfectly—hardly at all.]

Perhaps the best way to study the two series of photomicrographs compared above, and Photo's 23 and 24, is to attend chiefly to the bright lines and areas : to consider the positive effects of aperture in relation to the bright boundaries of bright areas, the bright boundaries of fields surrounding dark areas, the bright bands and bright spaces between dark bands ; and, on the other hand, to regard the dark areas and bands as negative interruptions having only corresponding interrupting effects in the projected image. "Drowning" effect would tend to weaken the intensity of the image of dark lines and particles, and to narrow images of broad areas of black. In critical micrometry it may be necessary not only to have a regard to aperture, but also as to the positive or negative character of the picture.

Experiment 9 : The general arrangement of the apparatus remained unchanged. An aërial image of the lines of Fig. 4 was projected by means of a Powell and Lealand achromatic sub-stage condenser of ⅓-inch focus, using dia-

phragm opening "3." The resulting aërial image was photographed with a Powell and Lealand ½-inch objective and 2-inch eye-piece to get Photo. 27. Photo. 28 was then taken after making but one change in the arrangement. Diaphragm opening "3" of the condenser was changed for the smallest, "1." Resolution fell off proportionately. Several diffraction lines may be seen in Photo's 27 and 28. Photomicrographs might be produced to show that diffraction phenomena parallel with those we have already studied occur in images projected by the sub-stage condenser.

Early in this study a telescope lens was used as the objective of a gigantic microscope. In the present experiment a microscope objective [for the sub-stage condenser is essentially such] was used as that of a telescope. The two instruments behaved alike. Moreover when a transparent object resting upon the microscope stage is illuminated by an image of the source of light projected in the plane of the object, it appears that diffracted light may be a factor of illumination and that a change in openings of the sub-stage diaphragm may alter the character as well as the intensity of illumination.

[Compare, for a moment, Photo's 27 and 28 with Fig. 4, and note the difference between the original object, as shown in Fig. 4, and the trebly-projected images. What a falling off is to be seen in the final pictures! a result of imperfection in lens projection. As we have double projection in ordinary practice with the microscope, we may believe microscopic vision has nearly two-thirds of the imperfection seen in Photo's 27 and 28 as compared with Fig. 4 when correspondingly high-power eye-pieces are used.]

Experiment 10: A telescope was arranged as in Experiment 2. Instead of the paper diaphragm with a single slot, diaphragms with two or more slots were used in the hood. Emitting points uncovered by pairs of slots (or an isolated zone of aperture) in a telescope behaved as we have seen the corresponding apertures of a microscope objective behave in the projection of diffracted phenomena.

Experiment 11: Fine and closely-ruled lines were

observed while diaphragms with minute openings were held between the lines and the eye. The conditions were varied so as to convince one that the dioptric apparatus of the eye projects diffraction phenomena parallel with those previously studied in images projected by the telescope, microscope and camera objectives.

It is a matter of common observation that black articles look smaller than white articles of the same size. A filament of black carbon appears broader when incandescent in the electric lamp. The eye is not corrected for spherical and chromatic aberrations. These aberrations cause light areas to appear to encroach on dark ones. Our experimental study suggests that diffraction effects are also a factor in the phenomenon. The apparent spreading of light into darkness increases with the intensity of the light. The brighter a star, the larger it looks. Is not this phenomenon, "irradiation," to the retina essentially what "halation" is to the sensitized film of the photographic plate?

The optical imperfections of the eye and the effects of its aperture, the pupil, contribute with those of the instruments we have considered to project on the retina images differing to a varying degree from the original object.

That the effect of aperture is important in the eye-piece is shown by the influence variation in the size of the opening in the eye-piece cap has on resolving power, both photomicrographically and visually. The field-lens is functionally a part of the objective, the two projecting the image at the diaphragm between the field-lens and eye-lens. The eye-lens and the lens of the observer's eye are a projecting combination capable of projecting on the retina all the diffraction phenomena we have found other lenses project.

Experiment 12: Photo. 32 shows the lines of an Abbe test plate [the same shown, inverted, in Photo's 9 and 10, and described at page 337] taken when the last emitting surface of the objective used was covered with a diaphragm which had an eccentric opening 1 mm. wide and transmitted only such rays as had been previously diffracted in the plane

of the object by the lines cut through a film of silver. Photo. 33 was taken under the same conditions, excepting that the eccentric opening was 2 mm. wide. The effect of increase of aperture, narrowing lines and favoring resolution, when using only such rays as had been diffracted by the object may be seen in the indistinct resolution in the upper half of Photo. 33. This is more distinct in the photomicrograph. Photo. 34 was taken under the same conditions as Photo. 32, excepting that the slot 1 mm. wide transmitted central primary rays. Photo. 35 was taken with a slot 2 mm. wide, half central and half eccentric, transmitting through its central half primary rays and through its eccentric half diffracted rays. Since the half-tone reproductions illustrating this comparative experiment were made, the experiment itself has been repeated (visually) several times. As a result the writer finds that when Photo. 33 was taken the width of the slot was not wholly filled with diffracted rays. This means that the *effective* aperture was a little less than 2 mm. wide. *Aperture beyond that which is utilized, or effective, contributes nothing to resolving power.* An accidentally lessened aperture is thus shown to be responsible for the inferior resolution in Photo. 33 as compared with that in Photo. 35. Repetitions of the experiment show that under parallel conditions the same aperture gives the same resolution with either diffracted or primary rays. In other words: *Aperture affects diffracted rays from an object as it does primary rays from an object.*

Disregarding the accidental difference in resolution Photo's 32 and 33 differ notably from Photo's 34 and 35. Certain negative imperfections, as breaks in a line, seen in the lower pair are also to be seen in the upper, the pairs being alike in this respect. Certain positive faults—scattered small splashes of light, images of little irregular areas broken through the silver film—seen in the lower pair are not to be seen in the upper pair or are replaced in the upper pair by corresponding negative faults. The positive faults in Photo's 34 and 35 were projected by primary rays. No primary rays contributed to the formation of the images seen in Photo's 32

and 33, but only diffracted rays of limited range as to obliquity. The position of the eccentric opening in the diaphragm determined the necessary obliquity of the rays which could be transmitted. The closeness of the lines in the silver film determined the obliquity of the diffracted rays which reinforced one another [diffracted rays of other obliquities leaving the lines in the silver film nullified one another by interference]. The position of the eccentric opening in the diaphragm was such as to transmit rays of the obliquity determined by the lines in the silver film. Certain positive faults of various breadths in the silver film gave rise to diffracted rays which reinforced one another at various obliquities outside the grasp of the eccentric opening in the diaphragm. Such rays could not reach the picture. Their absence resulted in negative "splashes" in Photo's 32 and 33 corresponding with certain positive "splashes" in Photo's 34 and 35.

There are objects which may be satisfactorily illuminated with a spot-lens or a sub-stage paraboloid—or by oblique light obtained in any way—so that no direct rays reach the objective, but only those changed in direction as by refraction or diffraction. Under such circumstances and in the case of a uniformly fine structure, for instance, the cone of rays taking paths to the objective might be exclusively diffracted light. Thus the image would be projected wholly by diffracted rays which on passing through the instrument would be affected by aperture as we have seen primary rays are affected by aperture. There would be one peculiarity about the intensity of such light in the cone between the object and the objective. The diffracted rays of greater intensity would be nearer the periphery; because the primary rays, from which they would originate, would be outside the cone. The rays would lose intensity with their nearness to the axis. [Diffracted rays originating from axial rays, as has been true in all previous instances cited, lose intensity in the opposite direction, or with their obliquity to the axis. This difference in the two kinds of cones may be shown to have some perceptible

influence in the projected image.] The possibility of such illumination is confined to the exhibition of objects observed under low power objectives and belongs rather to the æsthetic than to the practical domain of microscopy.

If we consider opaque objects illuminated for microscopical observation, all the rays between the object and objective are indirect—by reflection. These indirect rays can travel only in the paths already considered and can be affected in no new way by aperture. Has it not become evident that the diffraction of light by an object may be considered in the same category with other changes in direction in incident light produced by an object, as, for instance, those resulting from reflection?

In taking Photo. 10, between the object and objective was used a cone which consisted of a full cone of primary rays and a full cone of diffracted rays uniting at the origin of the latter in the object to form a compounded resultant full cone. In the case of a similar but coarser object, illuminated by transmitted light, the primary rays might be confined [first] to peripheral paths, or [second] to axial paths in such a cone. In the [first] instance: there would be a full cone of diffracted rays originated by the lines of the coarser object which, if alone, would be affected in no new way by aperture; and in addition, primary rays uniting with the peripheral diffracted rays. If the apertures at lens surfaces were not to be operative later, the only final result, in a projected image, of the increased intensity in the periphery of the cone would be a greater brilliancy of the picture. But both the faint axial and the more intense peripheral rays are affected by aperture, the latter rays tending to produce the phenomena we have seen result from projection by a zonular aperture. If the fainter axial rays were to have the same focus, they would tend to correct the false diffraction effects [obtruding zonular aperture effects] in the picture, by giving origin at the emitting surface of the objective to secondary rays, from secondary rays, tending to complete the diffraction effects of a continuous full aperture. In

the [second] instance: there would be the full cone of diffracted rays; and in addition, primary rays uniting with the axial diffracted rays. If the apertures at lens surfaces were not to be operative later, the only final result in the projected image of the increased intensity in the axial rays would be a greater brilliancy of the picture. But both the faint peripheral and the more intense axial rays are affected by aperture, the latter rays tending to produce the effects we have seen result from projection by a small circular aperture. If the fainter zonular rays were to have the same focus, they would tend to correct the defects [obtruding lesser aperture effects] in the picture, by giving origin at the emitting surface of the objective to secondary rays, from secondary rays, tending to complete the diffraction effects of a continuous full aperture.

In practice it has been found that when the general conditions of the [second] instance are made more specific, *when primary rays utilize a central circular surface of emission having a diameter equal to about three-fourths that of the full aperture, conditions are favorable for best microscopic vision*, more favorable than when the whole of the last surface is utilized by primary rays. Increased spherical aberration due to increased utilization of aperture has been supposed to explain why a full cone of primary rays does not give so perfect a picture as the three-fourths cone. Mr. E. M. Nelson* has shown that increased spherical aberration can only in part explain the difference. A fuller explanation for this at first sight contrary effect of a smaller aperture is needed, and may be given, as follows:

When the horizontal width of a 5 mm. square aperture resolved lines, as shown in Photo. 13, variation in the height of the aperture was found to have no influence on resolving power. A photomicrograph of the same aërial lines was taken with an aperture height of 2 mm.; and another, with a height of 8 mm. [the horizontal width remaining 5 mm].

* *The Journal of the Quekett Microscopical Club*, March, 1895, p. 30.

These photomicrographs only duplicated the result seen in Photo. 13.

Let us examine circular and square apertures in relation to the slots *4*, *2*, *1*, *3* and *5* of Fig's 6, 7 and 8. In Fig. 10 the slots *4*, *2*, *1*, *3* and *5* are shown bounded by full lines. The diameter of the circle *EFH* is three-fourths that of the outer circle *ABCD*. Let the area within the circle *ABCD* represent the last emitting surface of an objective. Let the object be a series of fine lines parallel with the length of the slots. Let *mnst* be a square aperture having a width equal to the diameter of the full circular aperture of the objective. The resolving power of a square aperture is about ten per cent greater than that of a circular aperture of the same width or diameter. The resolving power of the rectangle *opqr* is equal to that of the square, so far as the horizontal diameter of the objective is concerned [and this is the diameter upon which depends the resolution of the series of lines constituting our present object]. The other thirds of the square, the rectangular apertures *mnop* and *qrst*, have the same resolving power.

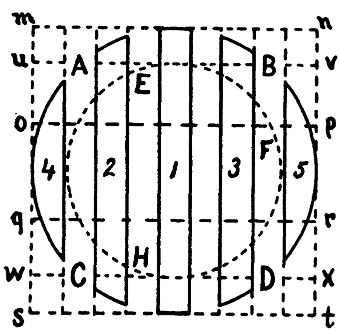


FIG. 10.

Now compare the portions of the slots within the middle rectangular aperture *opqr* with those within nearly the same area bounded by *op* above, *qr* below, and on the right and the left by the boundaries of the full circular aperture. The portions of slots *2*, *1* and *3* are the same. Those of slots *4* and *5* are nearly the same. If the upper and lower boundaries of the two areas were nearer, the portions of the slots *4* and *5* included in the area would be still nearer alike. If the upper and lower boundaries of the two areas were farther apart, the portions of the slots *4* and *5* included in the areas would be less alike. It becomes evident, then, that the portions of the circular and rectangular apertures between the lines *op*

and *qr* have nearly the same resolving power, and that, if the lines *op* and *qr* were somewhat nearer, the included portions of the two apertures would have practically the same resolving power.

Compare in a similar way the portions of the slots in the upper third of the square with the portions of the slots the corresponding part of the full circular aperture would utilize. The corresponding part of the full circular aperture would utilize almost the same portions of the slots *2*, *1* and *3* that are in the upper third of the square, but only comparatively small parts of the portions of the slots *4* and *5* that are in the same third of the square. What is true in this respect of the upper parts of the square and the larger circle is also true of the lower parts of the square and larger circle between the lines *qr* and *st*. The diffraction effects of the upper part of a full circular aperture are reinforced by the same effects of the lower part. These united effects tend to be those of an aperture corresponding to *2*, *1* and *3* rather than of an aperture corresponding to *4*, *2*, *1*, *3* and *5*. Then portions, as to vertical diameter, of the circular aperture tend to produce the effects of a continuous aperture between and including the slots *2* and *3* in Fig. *7*; and another portion, the effects of a continuous aperture between and including the slots *4* and *5* in Fig. 6. The result is intermediate aperture effects. In other words, a study of Fig. 10 gives us an explanation of the superiority in resolving power of a rectangular as compared with a circular aperture, a superiority we have already experimentally found to be about ten per cent.

Now, let the full cone of primary rays between the object and the objective be reduced one-fourth, to a three-fourths cone. The primary rays would then reach and fill the area within the circle *EFH*. Slots *4* and *5* would transmit no primary rays. Their diffraction effects in the image would be lost were it not for the fact that they would transmit diffracted light, diffracted by the object. The lines of the object being vertical, the diffracted light originated by them would be spread out horizontally to the left and the right, not vertically,

and would fill the horizontally opposite portions of the zone lying between the circles *EFH* and *ABCD*. The portions of the slots *2*, *1* and *3* above the line *uv* and below the line *wx* would not be utilized. The relative importance of slots *4* and *5* would be increased, by thus limiting the effect of aperture to the area between the line *uv* above and the line *wx* below. As a result, resolving power would be correspondingly increased.

Were the cone between an object and the objective to be so reduced that primary rays would reach and fill only a circular emitting surface having a diameter one-third of that of full aperture, the effective aperture would be limited above by the line *op* and below by the line *qr*. It would be approximately the equivalent of the rectangular aperture in relation to the horizontal resolution of the vertical lines. The same or more brilliant resolving power might be got by leaving the cone between the object and objective full and using behind the objective a diaphragm with a rectangular opening corresponding with *opqr*. By narrowing sufficiently the vertical width of the rectangular opening the resolving power might be made equal to that of the square aperture. This is not peculiar to the microscope objective. It is equally true of the telescope objective.* It is possible, however, for the accompanying loss of light from, for instance, faint double stars, or from faint details of microscopic objects, to more than counterbalance this increase of resolving power, visually [long exposure photographically here having an advantage].

With a narrow rectangular aperture resolution effected by the length of the aperture is limited to one diameter of an object. When, on the other hand, the cone between an

* It would be advantageous for some purposes, if practicable, to construct a narrow and rectangular astronomical telescope objective. Such a lens would possess nearly ten per cent more resolving power than a circular lens having a diameter equal to the length of the narrow lens, would be comparatively light in weight and require less strength of support. Or, with the same extent of surface as the circular lens and about the same amount of glass and weight, the length of the rectangular lens might be increased so as to transmit as much light and have several times the resolving power. The position of such a lens revolved about its principal axis to get best resolution would indicate the direction of a line uniting the components of a double star. In instances when the intensity of the light is unimportant, the surface of such a lens might be cylindrical instead of spherical.

object and the objective is reduced, the last emitting surface of the objective remaining uncovered, vertical lines have *a selective power*, as we have seen, utilizing horizontal aperture. In a similar way horizontal lines utilize a vertical aperture. Thus, any lines or details utilize appropriate aperture. "Selective power," if we may so call it, acts simultaneously and independently in different diameters, with a possibility of exhibiting details generally in an object ten per cent closer than the whole aperture filled with primary rays can exhibit. This is not peculiar to the microscope objective. It is equally true of any other lens projecting an image under parallel conditions.

As the Abbe theory requires a greatly reduced cone [for without such a cone diffraction by the object is nullified and "spectra" are absent], and as we have seen that a sufficiently narrow cone may so utilize the aperture of an objective as to make it the equivalent of a rectangular aperture in resolving power, we can account not only for the greater resolution of a three-fourths cone compared with that of a full cone, but for an excess of resolving power found in the Abbe numerical aperture tables.

Mr. Nelson has studied four kinds of illumination in practical work and finds the actual resolving power to be in the following order, the strongest first:

"Appearance at Back of Objective.

1. Peripheral annulus bright, $\frac{3}{4}$ center dark.
2. Peripheral annulus dark, $\frac{3}{4}$ center bright.
3. The whole dark (dark ground).
4. The whole bright (full cone)." *

Mr. Nelson finds this result not quite in harmony with theory. The difficulty may be explained by means of Fig. 10. The "peripheral annulus bright" is shown as the zone between the circles *ABCD* and *EFH*. In this zone are slots *4* and *5* and small portions of slots *2*, *1* and *3*. Because the preponderance of effective aperture corresponds with slots *4* and *5*, the resolving power of the circular aperture in this

* *The Journal of the Quekett Microscopical Club*, March, 1895, p. 34.

instance approaches nearly that of a corresponding rectangular aperture. The "peripheral annulus dark, $\frac{3}{4}$ center bright," has already been shown to have a resolving power greater than that of the full circular aperture and less than that of a corresponding rectangular aperture. Its resolving power is less than that of No. 1 and more than that of No. 4 of Mr. Nelson's list. On first thought, one would expect No's 3 and 4 to be equal in resolving power. We have already noted [page 369] a peculiarity of a cone of light leaving the object under the conditions of illumination present in No. 3, *i. e.* it is more intense at the periphery. This means that the effects of a peripheral zonular aperture are somewhat developed in No. 3. In No. 4 illumination is uniform or less intense at the periphery. So No. 3 is the equivalent of No. 4 plus a little of the effects of No. 1. Mr. Nelson's difficulty may be met thus without reference to the Abbe theory.

Great reduction in the cone of primary rays between an object and the objective in an attempt to make a round aperture approximate in resolving power a rectangular aperture is not advisable. The projected image loses dioptric intensity, by the loss of primary rays, in proportion to such reduction. Diffraction phenomena become more evident in the picture. False appearances may obtrude themselves in the image as a result of the rays diffracted by the object utilizing isolated zones of the objective, especially when the isolated zones of the objective have corresponding isolated foci.

Moreover, depth of focus—slight with a full cone of primary rays—increases with reduction in the cone. A full cone primary ray image is so nearly limited to a plane that it comes into or goes out of focus immediately—not gradually, while a narrow cone primary ray image responds to focusing so gradually that it is difficult to say when focusing is correct. With a full cone of primary rays of proper intensity we have seen that diffraction patterns are contracted to a minimum and may be visually absent. On the other hand, with a narrow axial cone of primary rays from such an object as our last, dif-

fraction patterns are broadened and multiple slot or zonular diffraction effects—due to the diffracted light from the object utilizing portions of the objective isolated from that utilized by the axial pencil—become pronounced in the image. The diffraction pattern may add force to a correct picture, or it may add false appearances such as we have studied in connection with Fig's 6, 7, 8 and 9, and shown in Photo's 2, 3, 4 and 5. It was noted that the diffraction pattern projected in Fig. 1 would appear on the screen if the screen were nearer or farther away from the sources of light. This is equally true of zonular diffraction patterns. Above and below the correct image plane are planes in which the zonular patterns may be focused. This phenomenon with the uncertainty of focusing correctly explains why an observer using too narrow a cone of light in illuminating an object is likely to see—by focusing isolated diffraction effects in other planes than that in which the primary ray is projected—diffraction "ghosts," and not the correct image at all.

Arrange such an object as closely-ruled fine lines for microscopic vision, illuminating it with a full or three-fourths cone of light from a sub-stage condenser. The bands of the primary ray image, all that can be seen, come into and go out of focus immediately, without gradually appearing or lingering. Now reduce illumination to a small axial cone. It becomes difficult to determine when the primary ray image is in focus. After the primary ray image is in focus, focusing upward or downward causes the picture to give place to imperfect repetitions of itself. Six or seven such repetitions, each succeeding one becoming more unlike the primary ray image, may be seen by focusing upward or downward. Such repetitions are diffraction effects, "ghosts" of the primary ray image; and while one "ghost" is changing into another more complicated diffraction patterns may be seen.

Theoretically and practically, then, we have seen that *advantageous reduction in a cone of light between an object and the objective may be made only within a limit. Beyond such a limit counterbalancing evils are likely to be met. The advis-*

able reduction in the case of a first-class objective is found to be from about one-fourth to one-third, never more than one-half, of the diameter of the cone.* The diameter and angle of the cone of light may be roughly determined and varied by means of a sub-stage mirror, or critically determined and varied by means of a sub-stage condenser of high quality, *i. e.* made with the same care given to the making of the best objective. *If so much depends upon the cone of light between an object and the objective, and this cone may be critically controlled only by means of a sub-stage condenser of high quality, is not one impressed with the advisability of habitually using a first-class aplanatic sub-stage condenser, and using it intelligently ?*

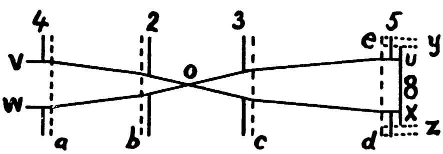


Fig. 11.

Experiment 13 : The general arrangement of the apparatus was the same as that used in taking Photo's 1 and 19. An opaque card, having a cross-shaped hole cut through it, was placed against the bull's eye condenser—instead of the card and holes used in taking Photo 1. An aërial image of the cross was projected in the plane of the microscope stage by a 1-inch objective, arranged as a sub-stage condenser. This aërial image was then observed through a $1\frac{1}{2}$-inch objective and a 2-inch Huyghenian eye-piece. Let Fig. 11 indicate diagramatically the relative positions of the sub-stage condenser, aërial image and objective. The dotted line *a* shows the position of the first lens of the sub-stage condenser, and *b* the position of the second lens of the sub-stage condenser. Let *c* show the position of the first lens of the objective, and *d* the position of the second or final lens of the objective. Let *4* represent the Powell and Lealand sub-stage diaphragm with circular opening "4" in use. Let

*See the description of Fig. 2 in the frontispiece of the Dallinger-Carpenter edition of The Microscope and its Revelations.

the angular lines *V O X* and *W O U* indicate the paths through the lenses which the boundary rays of the light transmitted by the diaphragm opening at *4* traveled. Experimentally it was found that a circular opening 2 mm. in diameter in a diaphragm capping the sub-stage condenser at *2*, a circular opening 3 mm. in diameter in a diaphragm capping the objective at *3*, and a circular opening 5 mm. in diameter in a diaphragm placed back of the objective at *5* just permitted all the rays transmitted by the diaphragm opening "4" to reach the emitting surface at *d*.

Then, without otherwise changing the arrangement, for diaphragm *5* with its opening 5 mm. in diameter was substituted diaphragm *8* [shown just back of *5*] which had a zonular opening and an opaque central portion 8 mm. in diameter. Diaphragm *8* obstructed all the primary rays emitted by the lens at *d*. The eye-piece was then removed. On looking at the back of the objective the zone *ed* uncovered by diaphragm *8* was illuminated and remained illuminated even when the circular opening at *4* was changed to "1" of the sub-stage diaphragm. The peripheral zone at the back of the objective was illuminated by rays which must have been separated from the direct axial rays at a previously operative lens surface.

The 2-inch eye-piece was replaced. The eye-lens of the eye-piece was removed. A 1-inch objective was mounted in the front board of an ordinary camera with the axis of the objective coincident with that of the microscope tube. The position of the camera with the objective was adjusted so as to project an image of the cross on the ground-glass of the camera with diaphragm *5* of Fig. 11 in place. The ground-glass was removed. In its stead was exposed a sensitized plate. The resulting photomicrograph is reproduced in Photo. 14.

Diaphragm *5* was then exchanged for diaphragm *8*. The field-lens of the eye-piece projected an aërial image of the annulus of light uncovered by the diaphragm *8*. This aërial image focused and photographed with the camera and its 1-inch objective is shown in Photo. 17. Immediately after-

ward, while observing the image of the annulus on the ground-glass as the camera with its 1-inch objective was pushed slowly towards the microscope, the annulus was seen to shrink gradually and become the small cross shown in Photo. 18, which is inverted as to the larger cross in Photo. 14. There can be no doubt, then, that the annulus was illuminated by light which in some way was derived from the aërial image of the cross projected in the plane of the stage, light which must have left the direct axial rays at some point in their paths. The inversion of the smaller image shows that the eccentric rays while traveling paths through the lenses were converged to at least one more focus than the direct axial rays. Such additional convergence was necessarily the result of at least two lens surfaces acting twice, each acting as a reflecting as well as a refracting surface. The exact paths taken by the eccentric rays have not been traced. It is probable, however, that they were separated from the direct axial pencil by internal and converging reflection at the emitting surface of the front lens of the objective and returned to the front surface, thence to be reflected back in an outward direction to and through the emitting surface along eccentric paths toward the annulus. Of what moment, separately or as a factor in compounded wave motion, the utilization of aperture by such faint aberrant rays is in microscopic vision does not here concern us. The writer shows that such rays are present chiefly because the same and similar rays have been regarded as factors in phenomena previously considered in our study [page 331 and page 363].

The Abbe "spectra" now invite our attention. We have found that they are not indispensable in the projection of some of the images of microscopic vision. "Spectra" are images of an opening in the diaphragm of a sub-stage condenser, or of a source of light, formed above the objective in the microscopic tube by diffracted rays originating in the object. Can an image of an opening, or of a source of light, formed in the microscope tube between the objective and the field-lens of the eye-piece by diffracted rays have any influ-

ence on the primary ray image of microscopic vision formed between the two lenses of the eye-piece? Two widely separated images cannot be seen through the microscope at the same time. In other words, such images cannot be united to form a joint visual picture.

The plane in which the Abbe "spectra" are seen, when present, is indicated by the dotted line *FF* in Fig. 2. The position of one of these "spectra" is indicated at the point in *FF* where the diffracted rays, dotted lines *vi* and *we*, cross. The rays of light in the paths *vi* and *we* leave the same point in the opening in the diaphragm of the sub-stage condenser as part of a primary ray pencil, diverge to the lenses of the sub-stage condenser, and are parallel on passing from the sub-stage condenser to the object *O*. As diffracted rays, a' v and d' w, they travel parallel paths to the lenses of the objective. From the lenses of the objective they converge to a focus in a plane *FF*. As to the object [now $a'd'$] the two diffracted rays leave its opposite extremities. As to the image of the object [now *i e*] they go to its opposite extremities. Of the innumerable rays which leave one point in the opening in the diaphragm and reach a corresponding point in the image of the opening in the plane *FF* no two pass through a common point in the object or arrive at a corresponding common point in the image of the object. On the other hand, each of the rays *v i* and *w e* is but one of countless rays which leave a common point in the object and reach a corresponding common point in the image on the screen. Then the image in the plane *FF* of an opening in the diaphragm of the sub-stage condenser is but an accident in the passage of light from the object to the eye. Its influence *as an image* in microscopic vision is nil. As an image it has no more to do with the image of microscopic vision than the image of Photo. 18 has to do with the image of Photo. 14, or than the image of a window frame has to do with the image of a distant hillside, each brought separately into view by focusing a telescope pointed through an open window.

When the cuts in a film of silver, shown in Photo's 9 and

10, were illuminated with a small axial pencil and a card held against the front lens of the objective one could see on the card, as already described [page 338], a round spot of light flanked on each side by fainter repetitions of the round spot. On taking away the card, removing the eye-piece and looking at the back of the objective one could see a picture similar to that previously seen on the card, a bright round spot flanked on either side by diffraction repetitions of the round spot, the latter in spectrum colors. These were in this instance images of an opening in the diaphragm of the sub-stage condenser, projected by the objective in the plane *FF* of Fig. 2. The lateral images in spectrum colors were the "spectra" of the Abbe theory, corresponding in number with the faint lateral repetitions of the round spot previously seen on the card. In explanation of Fig. 1 it was noted that light of different colors, with correspondingly different wave lengths, would produce interference phenomena at slightly different points on the screen. In a similar way the pencils of diffracted light brought to a focus in the plane *FF* have the rays of one color united at points a little removed from the points at which rays of another color are united; hence the spectrum colors in the diffraction images and the name "spectra." When the sub-stage axial pencil was changed to a full cone of light from the sub-stage condenser this picture in the plane *FF* was replaced by a large circular area of light, an image of the largest opening in the diaphragm of the sub-stage condenser, which image included and obliterated every indication of the small central spot and flanking "spectra." No "spectra" are to be seen back of the objective when an object is opaque and appears bright under ordinary conditions of illumination with reflected light. *So sometimes the Abbe "spectra" are present as an accident of microscope projection and sometimes they are not.*

Why do "spectra" when present appear to be so important? This apparent importance of "spectra" may be explained by means of Fig. 2. The "spectra" in the plane *FF*, near the back of the objective, are so situated that when a slotted diaphragm placed in the plane *FF* uncovers the

"spectra," under the conditions of Fig. 2, the same diaphragm uncovers simultaneously the emitting points *A*, *B*, *C*, *D* and *E* of the projecting lens. If now one of the slots of such a diaphragm be covered, one of the "spectra" disappears. A corresponding change occurs in the projected image. But this change is due to the loss of the slot and corresponding emitting point of the projecting lens, and not to the loss of one of the "spectra"; because, *if the "spectra" were absent, in full cone illumination, covering the slot in the diaphragm would produce the same change in the projected image.*

Let us compare Fig's 12 and 13. Fig. 12 shows paths certain primary rays travel from the source of light to the eye. Fig. 13 shows chiefly the paths certain diffracted rays of the Abbe theory travel from their origin in an object to the eye. In both diagrams let *C D* be a sub-stage condenser; *X Y Z*, an object corresponding with the object in Fig's 6, 7 and 8; *A B*, an objective; *V W*, a diaphragm having slots corresponding with [but supposed to be broader than] *4*, *1* and *5* in Fig's 6, 7 and 8; *a*, *i* and *q*, the primary ray image of microscopic vision; *F H*, a positive eye-piece; and *E*, the position of the opening in an eye-piece cap. Let *L*, *L* and *L* in Fig. 12 be light, from a somewhat distant source, which is focused at *Y* in the object by means of the sub-stage condenser. In Fig. 12 are shown above the object only such rays as leave *Y* and pass through the emitting points of the objective uncovered by slots *4*, *1* and *5* in the diaphragm *VW*. Such rays converge to a focus at *i* and then diverge towards the eye-piece, by which they are rendered nearly parallel so as to enable the eye to focus them upon the retina. In Fig. 13 *L* is a point in an opening of the diaphragm of a substage condenser, the diaphragm interrupting all but a narrow axial pencil of rays from the source of light. The primary rays leaving the point *L* are converged by the sub-stage condenser so as to be parallel. As parallel rays they pass through the object to the objective. By the objective they are focused in an image of *L* at slot *1*. They pass on each to a different point in the image *a*, *i* and *q*.

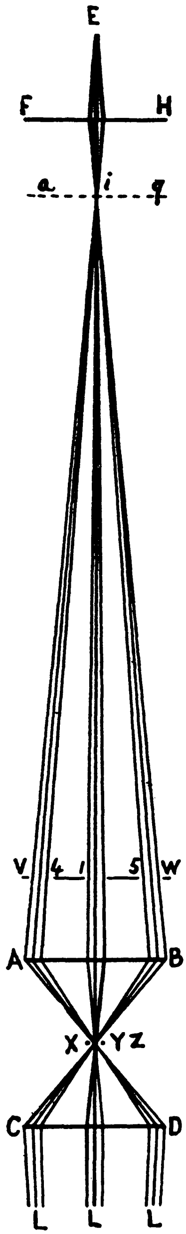


Fig. 12.

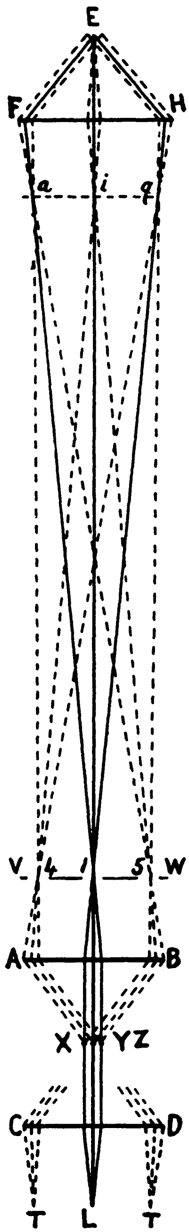


Fig. 13.

From the image *a*, *i* and *q* they pass to and through the eye-piece, by which they are focused in a second image of *L* at *E* in the opening of the eye-piece cap. From the image at *E* they diverge to such a degree as to make it *impossible for the eye to focus them upon the retina at the same time the rays shown in Fig. 12 are focused upon the retina.* The diffracted rays indicated by dotted lines have their origin in the plane of the object, and take such paths toward the objective as they would have taken if they had been primary rays starting from the points *T* and *T* in the opening in the diaphragm of the sub-stage condenser. The diffracted rays travel paths above the object similar to those just now described, and they fail to find a focus upon the retina for the same reason that the rays just now described fail to find a focus upon the retina. At slots *4* and *5* the diffracted rays form "spectra."

In Fig. 12, countless primary rays from *Y* are now supposed to pass through the slots *4*, *1* and *5* to form an

image of *Y* at *i*. In Fig. 13, of the innumerable rays passing through the diaphragm *VW*, three only—one primary ray passing through the slot *1*, one ray diffracted by the object and passing through slot *4*, and one ray diffracted by the object and passing through slot *5*—contribute to the projection of the image of *Y* at *i*. In Fig. 12, *a* and *q* would be projected as images of *Z* and *X* as *i* the image of *Y* is projected. In Fig. 13, the projection of the images *a* and *q* is indicated as being similar to the projection of the image *i* in the same diagram. Of the rays forming the image of the point *L* at the slot *1* in Fig. 13, one ray leaves *X* and goes to the image of *X* at *q*, one ray leaves *Y* and goes to the image of *Y* at *i*, and one ray leaves *Z* and goes to the image of *Z* at *a*. We see, then, by means of the diagrams that the image of the point *L* at slot *1* in Fig. 13 is but an accident in the passage of light from the object to the image of microscopic vision, *a*, *i* and *q*. The "spectra" formed at slots *4* and *5* in Fig. 13 are also seen to be but similar accidents in the passage of diffracted light from the object to the image of microscopic vision, *a*, *i* and *q*.

In addition to the image *a*, *i* and *q* in Figs. 12 and 13, there is projected a diffraction pattern, the union of the patterns indicated in lines *I* and *II* in Fig. 9, and shown in Photo's 30 and 31, as the result of slots *4*, *1* and *5* uncovering isolated portions of aperture. Suppose now that the diaphragm *V W* in Fig. 13 be removed. The primary rays and the diffracted rays would still travel the same paths. The same isolated portions of aperture would be utilized. The image and diffraction patterns of microscopic vision would be the same. Suppose, on the other hand, that the diaphragm *VW* in Fig. 12 be removed. Such rays of the full cone *CYD* as were previously interrupted by the diaphragm *VW* would now pass on to the image of microscopic vision, *a*, *i* and *q*. Every point in the emitting surface of the objective would be effective. The diffraction pattern previously seen would not be present, except possibly as residua—such as may be seen between *a* and *i*, or *i* and *q*, in Photo. 29.

Instead of using the word "spectra" as synonymous with images of an opening in the diaphragm of a sub-stage condenser projected in spectrum colors, let it be for a moment synonymous with rays diffracted from the object destined to reach one of the "spectra." Such rays would contribute to the projection of an image of an object just as primary rays would if the latter traveled the same paths. Both kinds of rays would form accidental images back of the objective of an opening in the diaphragm of a sub-stage condenser, or of a source of light under other but similar conditions. Neither kind of rays would have anything to do with the diffraction phenomena added to the dioptric image of an object were it not for aperture being effective in the ways we have previously studied. We have seen that the rays contributing directly to the diffraction phenomena added to a dioptric image are secondary rays originating at the emitting surface of the objective. That such secondary rays do not have their origin in the object may be shown by means of Fig. *7*. At *m* is a bright point in a diffraction pattern illuminated by rays which, if they had their origin in the object, would necessarily leave—to finally reach *m* in the image—a corresponding bright spot in the object half way between *X* and *Y*, *where there is no such bright point.*

When the axial illuminating pencil is narrow and the Abbe "spectra" are separated by well-marked intervals of darkness, the Abbe theory ignores the emitting surface of the objective corresponding with the intervals of darkness [*4–1* and *1–5* in Fig 13]. In harmony with this partial neglect of aperture, resolution in the Abbe theory may be said to increase by jumps. So long as a central image of the source of light alone is to be seen at the back of the objective [at slot *1* in Fig. 13] resolution is not present. The aperture may be increased without change in the contraction of the diffraction pattern, and in accompanying resolution, so long as the central image alone is to be seen at the back of the objective. But the moment the increase in aperture is sufficient to uncover, or admit, one flanking spectrum image [at slot *4*, or

slot *5*, in Fig. 13] resolution is present. With greater increase in aperture no improvement in the picture as to the contraction of the diffraction pattern, and as to accompanying resolution, is to be seen until another spectrum image is uncovered, or admitted.

On the other hand, with full cone illumination, resolution increases continuously, and not by jumps or by periodic accessions. The portions of aperture neglected in the Abbe theory, those corresponding with the Abbe intervals of darkness or the portions of the emitting surface of *A B* in Fig. 12 uncovered by removing the diaphragm *V W*, are effective in full cone illumination. They contribute in proportion to their breadth, radially from the principal axis, to the contraction of diffraction patterns. And thus they may resolve additional finer details [Experiment 14, below] in an object, or increase the distinctness of the resolution of details already resolved. Periodic accessions to resolving power are only observed when the particular conditions necessary to the Abbe theory, or similar conditions, are present. Again, then, we find the Abbe theory is unsatisfactory. On the other hand, the general explanation of resolving power in optical instruments is here applicable and satisfactory.

Experiment 14: A microscope was arranged to exhibit the lines shown in Photo's 10 and 35. For the optical part of a Powell and Lealand sub-stage condenser was substituted a Powell and Lealand 1-inch objective. A Powell and Lealand 3-inch objective and a Powell and Lealand "10 compensating" eye-piece were used. A diaphragm with an opening 10 mm. in diameter was placed at the back of the objective. The revolving diaphragm of the sub-stage condenser was turned so as to bring opening "1" into use. The closer lines of the test-plate were resolved. On removing the eyepiece and looking at the back of the objective, a central image of the opening in the diaphragm of the sub-stage condenser was seen flanked on each side at the limit of the aperture by about half of an Abbe spectrum image. The more distant halves of the "spectra" were just outside the limit of the aperture and could not be seen.

Then, for the diaphragm with an opening 10 mm. in diameter, at the back of the objective, was substituted a diaphragm having an opening 6 mm. in diameter. The latter just covered both halves of the two flanking "spectra," and left on each side of the central image a breadth of darkness corresponding with one portion of the aperture neglected in the Abbe theory. On replacing the eye-piece and again looking at the test-plate, the closer lines could not be seen. Resolution failed, because under the conditions present the Abbe theory requires for resolution the admission, by the diaphragm at the back of the objective, of at least a part of one spectrum image in addition to the central image of the opening in the diaphragm of the sub-stage condenser.

Again the eye-piece was removed. The diaphragm of the sub-stage condenser was turned so as to bring opening "3" into use. This change caused the central image seen at the back of the objective to increase in size until it filled the opening 6 mm. in diameter in the diaphragm at the back of the objective. On replacing the eye-piece and looking at the test-plate once more, the closer lines were seen. Resolution returned as a result of the additional light from the larger opening in the diaphragm of the sub-stage condenser reaching and utilizing the portions of aperture which were previously dark under the conditions necessary to the Abbe theory.

Our study of the Abbe "spectra" directs our attention to a relation existing between certain emitting points of a projecting lens, as D in Fig. 2, and the obliquity of rays diffracted by an object, as the obliquity of $a'v$ and $d'w$ in the same diagram. If the extremities of the object O, between the lines $b\,C$ and $c\,C$, were points of light in two isolated point-like holes in an opaque film of silver, the distance between them would measure a little more than a wave length of light, a little more than $r\,A$. Suppose the two holes were changed in position so that the upper one would correspond with a' and the lower one with the center of O. Let a similar hole, d', be placed as far below the center of O as a' is above the center of O. The object would then be three holes, with a distance

between the center of O and d' equal to that between the center of O and a'. Let parallel rays from the left be incident perpendicularly on the silver film in the plane $a' d'$. Primary rays passing directly through the holes towards C would be parallel to or in the principal axis of the objective. Then $a' v$, $O D$ and $d' w$ would be diffracted rays. The refaction of these rays by anterior lens surfaces between O and the emitting surface $A B C D E$ is not indicated. If the anterior lens surfaces were present and refraction shown, the three parallel diffracted rays, $a' v$, $O D$ and $d' w$, would have an obliquity of nearly 90° to the principal axis of the lens, in the case of a dry objective.

If the distance between the point-like holes subject to resolution were just equal to one wave length, the obliquity of the first effective diffracted rays leaving the object would be just 90° to the principal axis of the objective. If the distance were equal to two wave lengths, the obliquity of the first effective diffracted rays would be 30° to the principal axis; and then a second pencil of diffracted rays would be effective at an obliquity of 90° to the principal axis. If the distance were equal to three wave lengths, the obliquity of the first effective diffracted rays would be 19° 28′; and then a second pencil of diffracted rays would be effective at an obliquity of 41° 46′; and then, also, a third pencil of diffracted rays would be effective at 90°. With increasing distance between the point-like holes subject to resolution an increasing number of effective diffraction pencils of light would leave the object at obliquities of less than 90° to the principal axis of the objective, would pass through the objective, and would be emitted at points in the surface between C and D, or at points between C and B. If, on the other hand, the distance between the point-like holes were less than a wave length, and illumination were to remain axial, no effective diffracted rays would leave the object. All the diffracted rays about to leave the object would interfere one with another. Corresponding darkness would result at the front and emitting surfaces of the objective and in the

plane *F F*. Only the point *C* of the emitting surface would be effective. Only the primary ray axial image of the opening in the diaphragm of the sub-stage condenser would be projected in the plane *F F*. The angles of obliquity* of effective diffracted rays, to the principal axis of the objective, determined by the distances between the points of detail in an object are associated with certain points in the emitting surface of an objective, such as those indicated in Fig. 2, in a way similar to that in which they are associated with the Abbe "spectra."

In our study of aperture thus far we have had in mind illumination from a source of light situated in the principal axis of the projecting lens. Only for exceptional purposes, and only by those skilled in the manipulation of the microscope and in the interpretation of diffraction phenomena, should illumination from a source of light situated in a secondary axis of the objective be used. In all ordinary work the mirror or the sub-stage condenser should be placed centrally, the center of the mirror or the axis of the condenser coinciding with the principal axis of the objective. However, under proper circumstances the resolving power of an objective may be about doubled by using sufficiently oblique illumination from a source of light in a secondary axis of the objective. Let *O B* in Fig. 2 be a primary ray from a small source of light situated in a secondary axis of the dry lens of nearly 1.00 N. A., or nearly 180° in air, passing directly through a point-like hole at the center of *O* in the film of silver. Suppose parallel incident primary rays pass through similar holes at *a′* and *d′*. Such primary rays would leave the object at an obliquity of nearly 90° to the principal axis of the objective, if the anterior lens surfaces were present and refraction shown. As the first effective diffracted rays would leave the holes at an obliquity of nearly 90° to the direct primary rays the diffracted rays would take paths in the direction *O C*. Only about half

*The English translation of THE MICROSCOPE IN THEORY AND PRACTICE, by Professors Naegeli and Schwendener, p. 230.

the objective would be utilized. Thus we see that the obliquity of the first effective diffracted rays might have been twice as great, or double the effective aperture might have been utilized, or the holes in the film of silver might have been half as far apart.

If the holes had been half as far apart, the direct primary rays taking the same paths, the first effective diffracted rays would have had an obliquity of nearly 180° to the direct primary rays.* The direct primary rays, after passing through the objective, would have been emitted at *B*. The diffracted rays, after passing through the objective, would have been emitted at *D*. The emitting surface between *B* and *D* would have been dark, as the result of the corresponding diffracted rays interfering with one another. Thus, the points *B* and *D* would have been utilized as isolated points of emission. They would have behaved as we have seen points in an aperture uncovered by two widely separated slots behave. The resulting picture would have differed from that desired as Photo. 2, or 3, or 4, differs from Photo. 1. *Then Photo's 2, 3 and 4 are a pictorial warning for a second time, now a warning against the use of oblique illumination in ordinary work as a means of increasing or of attempting to exhaust the resolving power of the microscope. At the same time it becomes evident that every sub-stage should be provided with a means by which its condenser may be acccurately centered, and that every student using the microscope should be familiar with a method of centering his sub-stage condenser. These general rules should be accompanied by another: the sub-stage condeuser† should be as well made as the best objective, and should be used with an ever present appreciation of its power to improve or injure the picture of microscopic vision.*

It is theoretically barely possible by means of homogeneous immersion and the use of violet light to about double the resolving power of an objective, as compared with another of the same focus used dry with white light illumination.

* *Ibid.*

† Powell and Lealand "apochromatic" and "achromatic" sub-stage condensers are types of high quality. This cannot be said of the Abbe sub-stage condensers so commonly used in laboratory work. Consult the Dallinger-Carpenter edition of THE MICROSCOPE AND ITS REVELATIONS, from page 248 to page 263.

So, while BD in Fig. 2 indicates nearly the greatest aperture [nearly 180° in air, or nearly 1.00 N. A., or nearly an effective diameter of a quarter of an inch in a $\frac{1}{8}$-inch objective] obtainable in a dry objective, AE in the same diagram indicates the greatest possible aperture theoretically obtainable with ordinary objectives by the use of homogeneous immersion and shorter wave lengths. With the use of extremely oblique light we have found that resolving power in a given objective is about double what it is with principal axis illumination. Thus, with oblique light the aperture AE should theoretically resolve points or lines about a quarter of a white wave length apart, or about 190,000 points or lines to the inch. The extreme limit of resolving power shown in the Abbe numerical aperture table is 193,037 lines to the inch. [In this paragraph the circular aperture is supposed to be the equivalent in resolving power of a corresponding rectangular aperture.]

Practically, under the conditions suitable for best microscopic vision [p. 371], the theoretical limit of resolving power found in the Abbe numerical aperture table cannot be realized. The extreme theoretical limit of the table is that which would be "about on the point of" realization with a rectangular aperture and with the use of extremely oblique illumination and violet light. But in practice we use a circular aperture and—under the conditions suitable for best microscopic vision—principal axis illumination. Moreover, Mr. Nelson* finds that objectives transmit violet light only feebly. He also finds that while the use of blue light increases the resolving power of narrow aperture objectives it gives no better results as to resolution than the use of white light in the case of a wide aperture objective. For these reasons, any example of actual resolution noted in the first column of the Abbe numerical aperture table is much less than the corresponding theoretical resolution found in another column of the table.

1.00 N. A. is the equivalent in resolving power of an aperture of 180° in air. Therefore, in harmony with our

* *Journal of the Royal Microscopical Society*, 1893, p. 15.

study of a rectangular aperture of 180° in air, from page 388 to page 391, 1.00 N. A. should with principal axis illumination resolve points or lines one wave length apart. In harmony with our study of the same aperture with the same illumination, at page 340, points or lines half a wave length apart should "be about on the point of resolution" by 1.00 N. A. Then, in harmony with both these results, our theoretical limit of resolving power for 1.00 N. A. in the case of a rectangular aperture should be the resolution of points or lines less than one wave length apart and more than half a wave length apart, or a number of lines to the inch between 46,666 and 93,333, a wave length of white light measuring $\frac{1}{46666}$ inch. The exact number of lines an observer may see through an objective of 1.00 N. A. is uncertain. Varying keenness of vision is a factor in the uncertainty. Another factor in the uncertainty is the varying distinctness of the image of each of the points or lines subject to resolution, due to peculiarities* in the correction of the aberrations of the objective or to the use of monochromatic light for illumination.

The results actually obtained by Mr. Nelson place the limit of resolution for 1.00 N. A. with white light and principal axis illumination at about 70,000 lines to the inch. This result was obtained with a three-fourths cone of light between the object and the objective and with a circular aperture. It is interesting to note that 70,000 is three-fourths of 93,333 or that 70,000 stands just half way between 46,666 and 93,333. We may say that in practice 1.00 N. A. resolves points or lines a wave length and a half apart. By referring to Fig. 10 we see also that it may be said that a circular aperture should thoroughly resolve, in ordinary correct use of the microscope, the details of an object which would "be about on the point of resolution" by a square aperture having a diagonal equal to the diameter of the given circular aperture; for the diameter of the circle *A B C D* is a little less than the diagonal of the square bounded above by

* *Ibid*, p. 10.

uv, below by *wx*, on the left by slot *4* and on the right by slot *5*, the breadth of the square being equal to three-fourths of the diameter of the given aperture. If the limit of actual resolution be about 70,000 lines to the inch for 1.00 N. A., the limit for 1.40 N. A., nearly the highest numerical aperture in common use, is about 98,000 points or lines to the inch.

The more important results of our experimental study of aperture may now be summarized. It appears that diffracted rays leaving an object may be considered in the same category with other rays changed in direction by an object and that the diffraction phenomena seen in a projected image are essentially the effect of changes in light above the objective due to a function of aperture, and not to changes below the objective due to diffraction of light in the plane of the object. It also appears, however, that diffraction in the plane of the object does, under some circumstances, furnish light to certain parts of an aperture from which primary rays are absent and thus enables aperture to more fully determine the character of the projected image—resulting in a more nearly truthful image or, on the other hand, in false appearances: this is the gist of the Abbe phenomena of microscopic vision. But it appears, too, that such phenomena are not peculiar to microscopic vision, notwithstanding Prof. Abbe's claim to the contrary. Moreover, with any positive lens similar and more brilliant results may be got by utilizing corresponding isolated pencils of primary rays instead of isolated pencils of diffracted rays. Still more trustworthy results may be got by using continuous apertures three-fourths [in diameter] full of primary rays instead of the isolated pencils of primary rays.

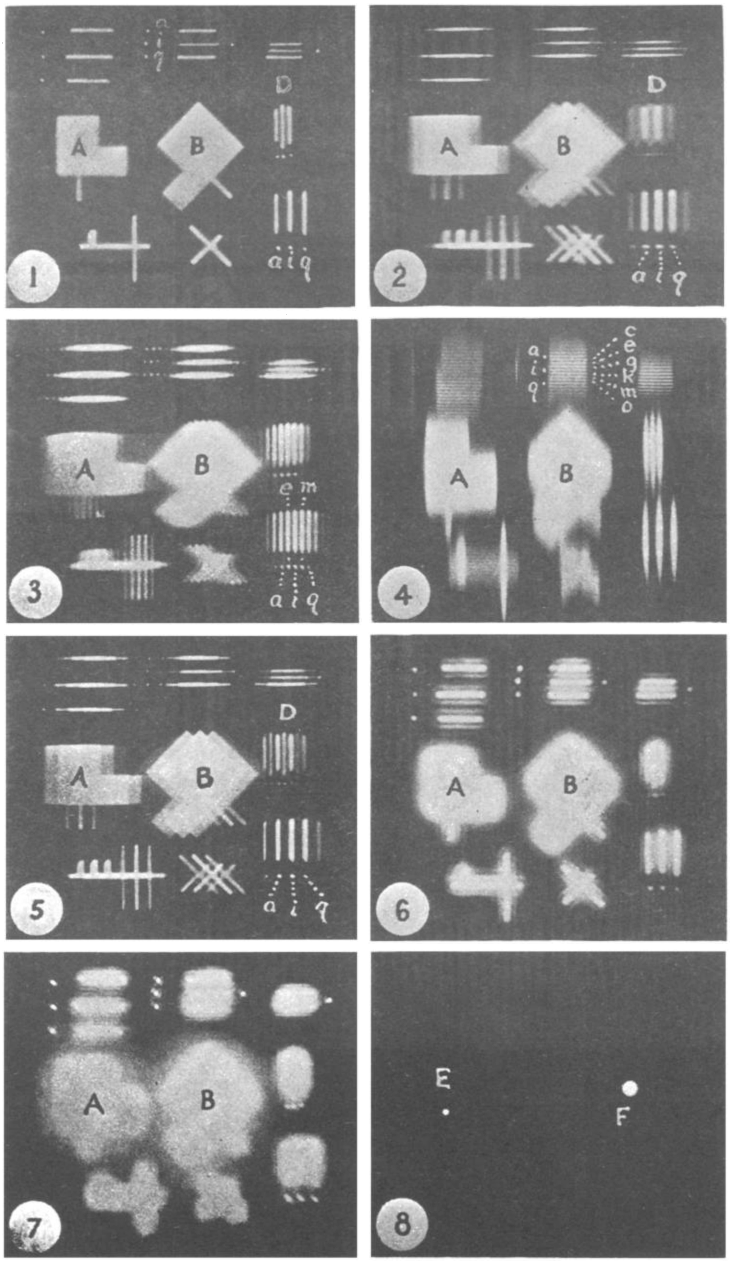
An advantage peculiar to using narrow cone illumination, the only illumination admissible in the Abbe theory with its indispensable "spectra," with an objective of wide aperture has received particular attention in this paper [for the first time, so far as the writer knows]. It has been shown that a circular aperture diaphragmed down to be

approximately the equivalent of a narrow rectangular aperture and filled with primary rays gives the acme of trustworthy resolving power possible in the corresponding diameter of the objective. But resolving power is reduced in all other diameters. Images projected by rays passing through such an aperture would be distorted essentially as the images of the dots *a* and *b* in Fig. 3 are shown to be at a' and b', or at a''' and b'''. It has been shown that by means of narrow cone illumination and an objective having a wide and uncovered aperture it is possible under suitable conditions to get approximately the acme of resolving power simultaneously in each of several diameters as a result of our so-called "selective power," the above distortion being incidentally eliminated. *Thus a circular aperture is approximately squared or made rectangular as to resolving power in several of its diameters simultaneously.* This approximate equivalent of "squaring the circle" as to resolving power is the advantage referred to as being peculiar to narrow cone illumination with an objective of wide aperture.

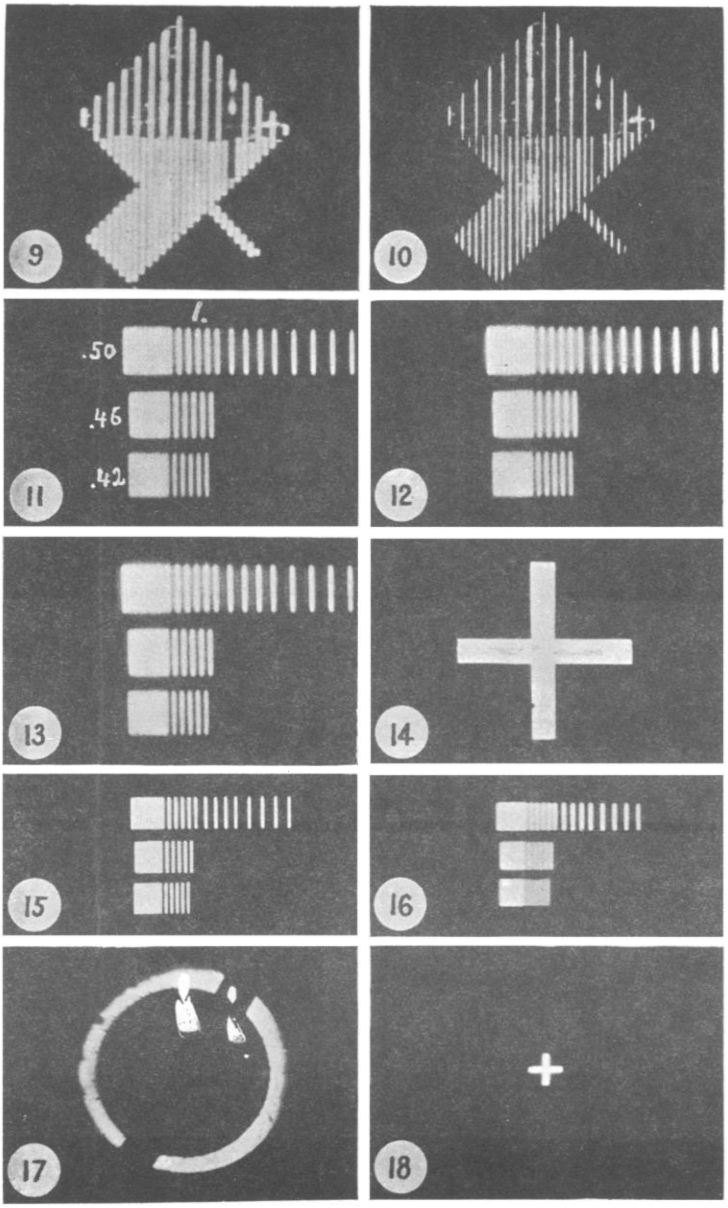
Finally, special attention is called to the fact that the Abbe theory deals with complex objects; for only such objects are subject to resolution. Single particles and uniform areas are outside its domain. These latter, however, are microscopic objects; and all objects are essentially different shaped aggregations of points. The visibility of an isolated point-like particle resembles the visibility of a star. No matter what the distance of the star, it may be seen if it has sufficient intensity. In a similar way, an isolated point-like particle—no matter what its minuteness—may be seen if it present sufficient contrast with the surrounding microscopic field. The size of the disc image in either case is no less than a limit determined finally by aperture. That limit in size, varying inversely with aperture, determines the limit of resolving power. This is the gist of the theory of microscopic vision which harmonizes with our experimental study of aperture.

It has seemed to the writer that an attempt to explain the

projection of an image of a complex microscopic object while ignoring the projection of the images of the points of which all objects may be considered aggregations resembles an attempt to explain, for example, the functions of a complex organism while ignoring the functions of its cells. An opposite course is more natural. In harmony with this more natural course the writer has attempted to present to those who may be interested an experimental study of aperture as a factor in microscopic vision.



PHOTO'S 1 TO 8 - REPRODUCTIONS OF PHOTOMICROGRAPHS



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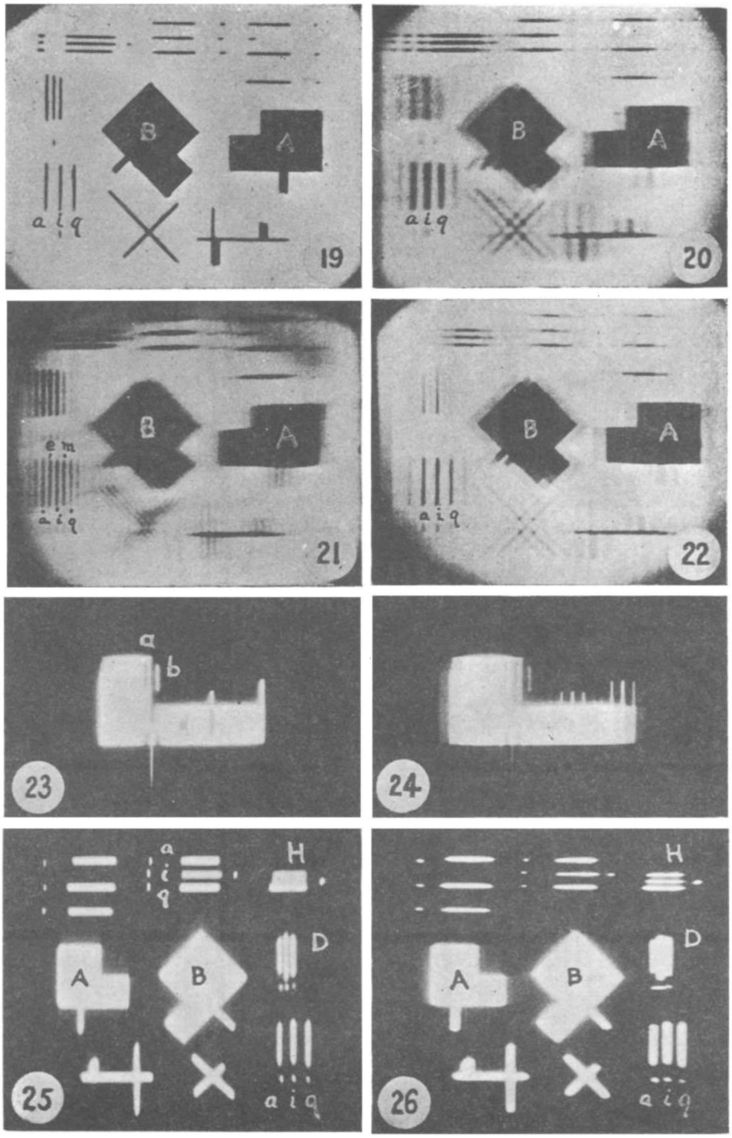
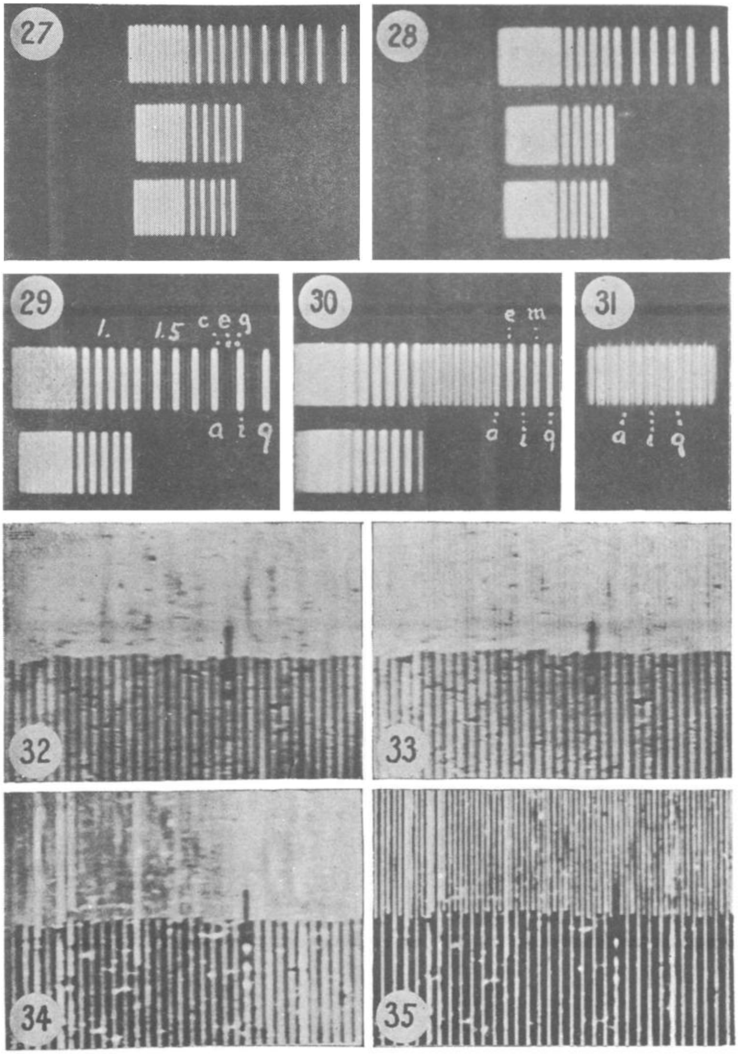


PHOTO'S 19 TO 26 - REPRODUCTIONS OF PHOTOMICROGRAPHS



PHOTO'S 27 TO 35 - REPRODUCTIONS OF PHOTOMICROGRAPHS